Journal

OF THE

ELISHA MITCHELL Scientific Society

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The Elisha Mitchell Scientific Society

Founded in 1883 through the efforts of Francis Preston Venable

H. D. CROCKFORD, President

W. R. STRAUGHN, Vice-President

E. T. BROWNE
Corresponding Secretary

W. A. BOWERS
Recording Secretary-Treasurer

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JOURNAL

OF THE

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PROCEEDINGS OF THE FORTY-EIGHTH ANNUAL MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

The forty-eighth annual meeting of the North Carolina Academy of Science was held at Duke University, Durham, N. C., on May 4 and 5, 1951.

The Academy convened in a general session at 10:00 a.m. on the first day with President A. S. Pearse presiding. At this session a Symposium on the Resources of North Carolina was presented.

The business meeting of the Academy was held at 4:30 p.m., at which the following business was transacted:

The minutes of the 1950 meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society **66**: 93–120.

Report of the Executive Committee

The Executive Committee met in Durham, N. C., on May 3 and 4, 1951. At these meetings the following matters were transacted:

1. The committee voted to pay the Mitchell Journal $400.00 for this year. This sum is to help defray the expenses of publication which have risen considerably in recent years.
2. It was voted unanimously to increase the annual membership dues to $2.50. The additional 50 cents is to be paid to the Journal of Elisha Mitchell Scientific Society. The present annual subsidy of $300.00 is in addition to the above mentioned 50 cents per member.
3. The secretary was authorized to do whatever he deems advisable to increase the membership.
4. It was voted to extend to colored scientists the professional privileges of the Academy.
5. In recognition of their outstanding contributions and service to the field of science the following were elected to Life Membership:

   George Thomas Hargitt, Duke University
   Leon Franklin Williams, North Carolina State College

6. The following were elected to membership in the Academy:

   Adams, David Arthur, N. C. State College
   Amore, Thomas, Cardinal Products Co., Durham
   Anderson, Carl Elmore, Biochemistry, School of Medicine, U. N. C.
   Balester, Raymond James, Psychology, University of North Carolina
   Balken, Eva Ruth, Clinical Psychology, Bowman Gray School of Medicine
   Bennett, Stelmon Emerson, Entomology, N. C. State College
   Bettinger, D. J., Chemistry, Davidson College

Blanton, Marion Elizabeth, Central High School, Charlotte
Bond, Austin DeMell, Science, East Carolina College
Bradford, Charles Robert, N. C. State College
Brown, Joshua Robert C., Zoology, Duke University
Brown, Robert Milton, Zoology, N. C. State College
Carr, Noel Cleveland, Science, Atlantic Christian College
Charles, James Robert, Zoology, N. C. State College
Cheek, Paul H., Chemistry, Elon College
Cobb, William Battle, Jr., Soil Conservation Service, Raleigh
Cole, B. Theodore, Physiology, Duke School of Medicine
Collins, Mrs. E. R., Chemistry, Meredith College
Conway, H. McKinley, Jr., Southern Association of Science and Industry, Atlanta, Ga.
Copeland, Margaret E., General Science, East Durham Junior High School
Corbin, Frederick Thomas, Wake Forest College
Cummings, Edmund George, Zoology, N. C. State College
Eaton, Theodore H., Jr., Science, East Carolina College
Farrier, Maurice Hugh, Entomology, N. C. State College
Fulton, Lewis McLeod, Jr., Mathematics, Duke University
Gafford, Elma Jackson, Science, Boyden High School, Salisbury
Golby, Robert L., Biochemistry, School of Medicine, U. N. C.
Goldie-Smith, Elizabeth Kathleen, Botany, University of North Carolina
Golin, Edward, Psychology, University of North Carolina
Hebert, Teddy T., Plant Pathology, N. C. State College
Heron, Stephen Duncan, Jr., Geology, Duke University
Irvin, Joseph Logan, Biochemistry, School of Medicine, U. N. C.
Jenner, Charles Edwin, Zoology, University of North Carolina
Johns, Robert M., Botany, University of North Carolina
Johnson, Sidney Pickette, Botany, University of North Carolina
Kamen-Kaye, Maurice, Consulting Petroleum Geologist, Chapel Hill
Kinkead, Alice Marcine, Meredith College
Koch, William Julian, Botany, University of North Carolina
Kritzler, Henry, Duke University Marine Laboratory, Beaufort
Lecka, George Mitchell, Science, Boyden High School, Salisbury
Lecka, Louise B., Science, Boyden High School, Salisbury
Leiserson, Lee, Liggett and Myers Tobacco Co., Durham
Locklair, Earl Everett, Durham
McClain, Elmo Fondren, Entomology, N. C. State College
Mayo, Bruce Roland, Mathematics, N. C. State College
Nevin, Charles S., Chemistry, N. C. State College
Newell, Edmond Bruce, Biology, Catawba College
Patton, Ernest G., Botany, Duke University
Payton, Carolyn Robertson, Psychology and Education, Livingstone College
Peeler, Ellen Lillian, Meredith College
Penrod, Kenneth Earl, Physiology and Pharmacology, Duke University School of Medicine
Pittard, Joanna Catherine, Meredith College
Ratledge, Hayden Monroe, Fisheries Investigator, Marion
Raver, D. F., Wildlife Resources Commission, Raleigh
Roberts, John H., Mathematics, Duke University
Ryan, Alice Jean, Woman's College U. N. C.
Saylor, J. H., Chemistry, Duke University
Sandeen, Muriel Ione, Zoology, Duke University
Schoenborn, Edward Martin, Jr., Chemical Engineering, N. C. State College
Shaeff, Charles B., Jr., Phipps and Bird Inc., Richmond, Va.

Shepard, Frederick Carlyle, Psychology, University of North Carolina
Silva, Joseph Andrew, Mathematics, Duke University
Stacy, Emily Mildred, Botany, University of North Carolina
Stewart, Margaret McBride, Woman's College U. N. C.
Sullivan, Robert Little, Zoology, N. C. State College
Tanner, Paul Eugene, Mathematics, Atlantic Christian College
Taylor, Harden F., 115 Broadway, New York, N. Y.
Tove, Samuel B., Animal Industry, N. C. State College
Van Bavel, Cornelius H. M., Agronomy, N. C. State College
Van Cott, Harold Porter, Psychology, University of North Carolina
Walker, Thomas Banner, Chemistry, Duke University
Weisman, Donald Marion, Entomology, N. C. State College
Weybrew, Joseph Arthur, Agronomy, N. C. State College
Whittinghill, Maurice, Zoology, University of North Carolina
Wilson, Dorothy, Durham High School
Wilson, John Eric, Biochemistry, School of Medicine, U. N. C.

7. The following were reinstated as members in the Academy:

Amick, Thomas C., Louisburg College
Dearborn, Donald C., Catawba College
Hickerson, Thomas F., Mathematics, University of North Carolina
Hill, Douglas G., Chemistry, Duke University
Perry, Harold Sanford, Botany, Duke University

8. The Secretary reported the following losses in membership:

1. By resignation: 5
2. By non-payment of dues: 55
3. By death the following:
   Mychyle Wellington Johnson

The above recommendations and reports were approved by the Academy

TREASURER'S REPORT

Financial Statement as of September 1, 1951

Receipts	
Bank balance (from July 1, 1951)	$290.73
Gifts	
AAAS Research Grant, 1950	91.00
AAAS Research Grant, 1951	96.50
John Bewley Derieux Memorial Award	50.00
Ornithology Essay Award	20.00
N. C. Section of A. C. S., 1949, 1950, 1951	15.00
J. D. Ives	5.00
Dues, 1950	8.00
Dues, 1951	846.00
Total	1422.23
Expenditures	
Journal of Elisha Mitchell Scientific Society	400.00
Secretarial aid	130.00
Secretarial commission	84.00
AAAS Research Grant, 1950 to R. K. Godfrey	91.00
AAAS Research Grant, 1951 to Maurice Whittinghill	96.50

Stationery, printing, programs (N.C. State Print Shop)	92.96
Postage and stamped envelopes	69.53
High School Student Awards	60.00
John Bewley Derieux Memorial Award to Judson Hardy, Jr.	50.00
Reprints (Waverly Press)	12.88
Meeting expenses	11.75
Office supplies	11.30
Express charges	7.88
Bank charges	7.51
Derieux Committee (Anna Joyce Reardon and Piedmont Press)	6.20
Telephone (long distance calls)	2.56
Total	1134.09
Bank balance	248.16
Cash and checks on hand	40.00
	1422.23

Savings Account

Balance (from July 1, 1950)	100.00
Interest	3.02
Total	103.02

Total Assets

Checking account and cash on hand	288.16
Savings account	103.02
U. S. savings bonds (maturity value)	300.00
Total	691.18

On the twenty-first day of September, 1951, we, the undersigned, examined this account and found it to be correct.

(Signed) H. M. NAHIKIAN
R. C. WHITE
J. S. MEARES, *Chairman*

REPORT OF THE RESEARCH GRANTS COMMITTEE

The grant of $91.00 for the past year was given to Mr. R. K. Godfrey of N. C. State College. The grant was wholly expended in partial defrayment of travel expenses involved in field work relating to a study of North Carolina Compositae during the 1950 collecting season.

RUTH M. ADDOMS
A. D. SHAFTESBURY
W. O. PUCKETT, *Chairman*

THE POTEAT AWARD

The committee decided to grant the award to Mr. Steve G. Boyce of N. C. State College for his excellent paper, "Source of atmospheric salts."

W. B. FOX
REINARD HARKEMA
H. B. HOYLE, JR.
MARION STANLAND
D. S. GROSCH, *Chairman*

After considerable debate the Academy passed the following motion with reference to the Poteat Award: That the Poteat Award be rotated among the sections in alphabetical order, provided there is a paper meritorious of the award. If no paper in the particular section meets the requirements of the award, the latter will be selected from the section next in line. Further, any paper appropriate for presentation before the "award section" but read before the general session will also be eligible.

The selection of the winner is to be made by the members of the "award section" present at the reading of the papers.

REPORT OF THE CONSERVATION COMMITTEE

The committee is pleased to report that two recommendations made to the Academy last year have been acted upon by the Legislature—those dealing with protection of the Venus' fly-trap and with stream pollution.

Members of the Conservation Committee met with the Legislative Committee and with the Director of the North Carolina Department of Conservation and Development, George Ross. Plans were made for the introduction of a bill to protect the Venus' fly-trap. Mr. Ross had the staff members of his Forestry Division prepare a bill along the lines recommended by the two committees.

Mr. Harry Mintz, Representative from Brunswick County, introduced H. B. No. 373 in the House and Senator Brink from Brunswick County introduced it in the Senate. Members of our committee supplied these two legislators with information and pictures of the sale of the plant. Both houses passed the bill and it is now law.

The law provides that any person, firm, or corporation found selling or exporting the Venus' fly-trap shall be guilty of a misdemeanor and shall be fined or imprisoned in the discretion of the court. It also provides that the plant can be sold and exported for purposes of scientific experimentation and study when such sale or export has been authorized in writing by the Department of Conservation and Development.

The committee did not have any direct contact with the sponsors of the stream pollution bill.

Our job now has just begun in that we will have to help see that the law is enforced. And we can do this very effectively. Each of us can be on the lookout for the sale and export of the plant. We can report infractions to the local authorities and to the N. C. Department of Conservation and Development. We can ask the other organizations, garden clubs, bird clubs, science clubs, college faculties, men's clubs, and school teachers to assist in reporting such sale or export.

We now have the legal machinery, so let's develop the interest and concern to make the law effective.

F. S. BARKALOW
W. D. MILLER
COY T. PHILLIPS
B. W. WELLS
R. L. WEAVER, *Chairman*

The report was accepted by the Academy.

REPORT OF THE LEGISLATIVE COMMITTEE

This committee was given its assignment last May. The assignment came from the deliberations of the Academy Conservation Committee as follows:

1. To control the sale of, and thus conserve, the Venus' fly-trap plants.
2. To regulate introduction of exotic game species.
3. To appropriate sufficient money for an adequate forestry program.
4. To control and correct stream pollution.

On July 25, 1950, the Academy Secretary notified us of the assignment. Following this the three of us discussed the proposals informally among ourselves and with other interested persons.

In the conservation of the Venus' fly-trap the organized garden clubs were interested but not prepared to initiate legislation.

On the introduction of exotic species the Wildlife Resources Commission seemed to have a passive policy. Laws and regulations under the Veterinary Division of the Department of Agriculture require official reports and inspection of introduced animals, both exotic and not so. Enforcement of these requirements might serve the conservation purpose.

On forest conservation, there is a strong lay organization and we offered our support to appear before legislative bodies, if desirable.

On stream pollution, we found an active group and proposed legislation. We secured some 200 copies of these legislative proposals and your Secretary mailed them to selected Academy members who might win friends and influence action. We offered to provide supporters for legislative hearings.

On December 18, 1950, your committee met in Dr. Stuckey's office and decided to take specific action in protection of the Venus' fly-trap. We realized that this was a new field in interference with private control and exploitation of wild plants, so far as this state is concerned. After conferring with George Ross, Director of the Department of Conservation and Development, we decided to attempt to stop commercial traffic in this species. Dr. Weaver worked with Mr. Ross and his staff in the preparation of the bill which was introduced by Representative Mintz and Senator Brink from Brunswick County. The bill was passed and is now law.

The stream pollution bill was finally passed as a compromise measure. It will have to be tested before it can be known whether it will be of much help in reducing pollution.

Further study of exotic game species will be needed before definite action can be taken.

J. L. STUCKEY
R. L. WEAVER
H. T. DAVIS, *Chairman*

The report was accepted by the Academy.

HIGH SCHOOL CONTESTS

Announcement of the winners of the various high school contests was made by Miss Inez Coldwell as follows:

Forestry Essay Contest

(*Sponsored by the N. C. Forestry Association*)

"North Carolina Forests, Their Past, Present, and Future"
First Prize: Jimmie Lee Kesler, Rockwell High School, Rockwell
Second Prize: Mary Long, Belmont High School, Belmont
Third Prize: Jane Andrews, Mt. Olive High School, Mt. Olive
Honorable Mention: Jackie Hull, Salem High School, Morganton
Rebecca Riddick, Sunbury High School, Sunbury
Tommy Parsons, Belmont High School, Belmont

Ornithology Essay Contest

"Birds in Relation to Other Wildlife"
First Prize: William G. Cashwell, Mt. Olive High School, Mt. Olive
Second Prize: Nancy Burns, Lumberton High School, Lumberton
Third Prize: Boenita McCloud, Cranberry High School, Elk Park
Honorable Mention: Margaret Fields, Mt. Olive High School, Mt. Olive

Exhibits Contests

Group I. Living Plants or Animals (*Sponsored by Carolina Biological Supply Co.*)
First Prize: Ann Alexander, Central High School, Charlotte
"Hydroponics"
Group II. Preserved Plants or Animals (*Sponsored by Carolina Biological Supply Co.*)
First Prize: Sara Ruth Luckadoo, Morganton Senior High School, Morganton
"Flower Collection"
Honorable Mention: Sara Jo Hensley, Morganton Senior High School, Morganton
"Flower Pictures"
Group III. Practical Applications of Science
First Prize: Bennette Whisnant, Morganton Senior High School, Morganton
"Scientific Principles of Farming"
Group IV. Scientific Principles
First Prize: Bobby Ramsey, Central High School, Charlotte
"Stroboscope" and "Geiger Müller Radiation Counter" and "Accurate Beta Gamma Counter"

REPORT OF THE JOHN BEWLEY DERIEUX MEMORIAL AWARD COMMITTEE

The Committee met at 3:00 p.m. on March 31, 1951, at the Woman's College of the University of North Carolina. The committee decided unanimously to give the 1951 award to Judson Hardy, Jr., a third year student at the University of North Carolina, for his paper entitled *The Van de Graaf Electrostatic Generator.*

It is the hope of the committee that the members of the Academy will develop an interest in the John Bewley Derieux Memorial Award and will encourage the physics departments of their respective institutions to participate each year. In

this way interest in physics will be stimulated and other benefactors may be encouraged to establish similar awards for the other fields of science.

NATHAN ROSEN
O. J. THIES, JR.
ANNA JOYCE REARDON, *Chairman*

The presentation of the award was made at the evening meeting of the Academy.

REPORT OF THE RESOLUTIONS COMMITTEE

Be it resolved that the North Carolina Academy of Science express its sincere appreciation to Duke University, the Administrative officers, the faculty, and to all students concerned for the generous and gracious hospitality it has received and for the many courtesies extended it during this, the forty-eighth meeting of the Academy.

Be it further resolved that special appreciation be expressed to the local committee on arrangements whose careful planning of details has contributed greatly to the success and pleasantness of the meeting that has been enjoyed this year on the campus of Duke University.

Also, be it resolved that this report be spread upon the minutes of this meeting of the Academy and that copies be sent to President Hollis Edens and to Professor Paul J. Kramer for the Committee on Arrangements.

O. C. BRADBURY

The above report was unanimously approved by the Academy.

The following memorial was presented at the meeting:

MYCHYLE WELLINGTON JOHNSON

Mychyle Wellington Johnson, Associate Professor of Zoology at Duke University, died in his office September 25, 1950, at the age of forty. He was born at Mount Horeb, Wisconsin, September 11, 1910, the son of Mr. and Mrs. Thomas C. Johnson. His early childhood was spent in Wisconsin, but while still in grade school he was taken by his mother to Indiana where he completed his secondary education. He was able to work his way through Indiana Central College, and was granted the Bachelor of Arts degree in 1930. He held a graduate assistantship at the University of Indiana from 1930 to 1935 and in 1936 received the Ph. D. degree. It was at Indiana that he came in contact with such outstanding zoologists as Alfred Kinsey and Fernandus Payne. His chief work was with Dean Payne in the field of cytology with special reference to the nucleus.

In 1935 he married Mary Moore and joined the staff of the Carnegie Institute of Genetics of Washington station located at Cold Spring Harbor, Long Island. As research associate with Dr. Oscar Riddle he published several papers in the field of endocrinology and cytology.

In 1937 he came to Duke University as Instructor in Zoology and held successively the positions of Assistant Professor and Associate Professor. He was

a member of the American Society of Zoologists, the North Carolina Academy of Science, the American Association for the Advancement of Science, and was American collaborator for the International Institute of Embryology in Holland.

Dr. Johnson's scientific contributions were chiefly in the field of cytology, but within recent years he had developed a productive line of research on embryological abnormalities in the circulatory system. His last work on this subject received high praise from members of the American Association of Anatomists and members of the North Carolina Academy of Science.

Professor Johnson served his community and university well. He played in the percussion section of the North Carolina Symphony Orchestra, and in the Duke University Concert Band. He was one of the founders of the Duke-Durham Bird Club, and served as its president for a number of years. He liked to work with children and he was frequently called upon by members of the Durham Recreation Department. He worked hard to establish the Children's Museum on a satisfactory working basis. He served as treasurer and from 1948 to 1950 as secretary of the Duke Chapter of Sigma Xi.

Dr. Johnson's personal relationship with the students was exceptionally good. As a teacher of premedical subjects and as premedical adviser in zoology he came in contact with many undergraduates. They all spoke of him as an excellent teacher with high standards, but perfectly fair and considerate. He was always available for consultation and students confided in him.

As an associate he was always modest; his opinion and advice were frequently asked because of his sound judgment, broad knowledge, and lack of personal prejudice. He was always willing to do what he thought was the best for the student, the university or community, with no thought of self-glorification.

The premature death of our colleague is a great loss to the North Carolina Academy of Science. Our sympathy is extended to Mrs. Mary Johnson and her two sons, Jerry and Jan.

CAZLYN G. BOOKHOUT

The above memorial was approved by a rising vote.

REPORT OF THE NOMINATING COMMITTEE

The committee, consisting of J. W. Lasley, Jr., Chairman, F. G. Hall, and C. F. Korstian, submitted the following nominations:

President: W. C. George, University of North Carolina
Vice-President: Otto Stuhlman, Jr., University of North Carolina
Member of the Executive Committee: W. O. Puckett, Davidson College

The above nominees were unanimously elected by the Academy. This concluded the business and the meeting was adjourned.

President Hollis Edens of Duke University welcomed the Academy to the Duke Campus at the complimentary dinner given by the University. At 8:15 the evening session was held in the auditorium of the Biology Building with Vice-President E. L. Mackie presiding. The 1950 Poteat Award was presented to Dr. C. G. Bookhout of Duke University. The first presentation of the John Bewley Derieux Memorial Award was made by Dr. Anna Joyce Reardon to Judson Hardy, Jr.,

of the University of North Carolina. The winners of the various high school student contests were announced by Miss Inez Coldwell. President Arthur S. Pearse's entertaining and informative address on "Trying To Be A Zoologist" was enthusiastically received by the Academy. The members then adjourned to the lobby for an entertaining informal reception.

The following officers were elected for the respective sections:

Biochemistry and Physiology: Chairman, Maude Williams; Secretary, Marjorie Swanson

Botany: Chairman, R. K. Godfrey; Secretary, W. B. Fox

Geology: Chairman, Harry E. LeGrand; Secretary, E. W. Berry

Mathematics: Chairman, Anne Lewis; Secretary, H. M. Nahikian

Psychology: Chairman, Dorothy Adkins; Secretary, Marion Stanland

Wildlife: Chairman, Win Donat; Secretary, W. L. Hamnett

Zoology: Chairman, Maurice Whittinghill; Secretary, C. E. Jenner

The following constitute the personnel of the standing committees:

Executive: W. C. George, Otto Stuhlman, Jr., Reinard Harkema, C. H. Bostian, E. C. Cocke, W. O. Puckett

Research Grants: G. R. MacCarthy, A. D. Shaftesbury, W. O. Puckett, Ruth M. Addoms

Dr. W. C. George, President of the Academy for 1952, announced the appointment of the following committees:

Auditing: J. S. Meares, Chairman, H. M. Nahikian, R. C. White

Conservation: R. L. Weaver, Chairman, B. W. Wells, F. S. Barkalow, W. A. Chipman, R. J. Preston

High School: Christine Wilton, Chairman, A. D. Bond, J. O. Derrick, Mary Caughey Helms, R. M. Helms, W. A. Shannon

Legislative: F. A. Wolf, Chairman, R. L. Weaver, J. A. Yarbrough

Nominating: A. S. Pearse, Chairman, J. N. Couch, O. C. Bradbury

Resolutions: Mary Caughey Helms

Representative on A. A. A. S. Council: D. P. Costello

Representative to Academy Conference: D. P. Costello

Constitution: H. L. Blomquist, Chairman, H. R. Totten, Reinard Harkema

John Bewley Derieux Memorial Award: M. L. Braun, Chairman, F. W. Lancaster, Otto Stuhlman, Jr.

Approximately 350 members and guests registered during the meetings.

The following papers were presented during the meetings. Those marked with an x are abstracted in these proceedings:

Address of Welcome. President Hollis Edens, Duke University

Presidential Address: Trying to be a zoologist. A. S. Pearse, Duke University

SYMPOSIUM ON NORTH CAROLINA RESOURCES

Geological Resources. J. L. STUCKEY, State Geologist

Chemical Resources. E. M. SCHOENBORN, N. C. State College

Botanical Resources. F. A. WOLF, Duke University
Zoological Resources. R. E. COKER, University of North Carolina
The Economics of North Carolina Resources. H. F. TAYLOR, New York City

BIOCHEMISTRY AND PHYSIOLOGY SECTION

Carbon dioxide and tolerance to altitude. F. G. HALL and K. D. HALL, Duke University
x*Cardiac activity in hypothermia.* K. E. PENROD, Duke University
Studies in the calcification mechanism as influenced by fluorides. M. K. BERKUT and R. L. GOLBY, U. N. C.
Incorporation of labeled dimethylethanolamine into the lipides of surviving liver slices. MARIETTA CROWDER and CAMILLO ARTOM, Bowman Gray School of Medicine
Studies in the metabolism of C^{14}-labeled acetate. C. E. ANDERSON, U. N. C.
Preparation and metabolism of tyrosine disulfonate. J. C. ANDREWS and J. P. FEASTER, U. N. C.
Creating male or female children. H. F. PRYTHERCH, Beaufort, N. C.
Studies on liver pyrophosphatase. M. A. SWANSON, Bowman Gray School of Medicine
Surface tension determinations of some salurated fat acid methyl esters. C. S. NEVIN, N. C. State College
Variation in coloring rates of tobacco. J. A. WEYBREW and P. E. GREEN, JR., N. C. State College

BOTANY SECTION

Phytoplankton in fertilized and unfertilized ponds. L. A. WHITFORD, N. C. State College
A study of flagellation in Vaucheria. W. J. KOCH, U. N. C.
Preliminary taxonomic studies in Pluchea. R. K. GODFREY, N. C. State College
x*Further observations on the motile cells of* Actinoplanes. J. N. COUCH, U. N. C.
Cypress knees as aerating organs. P. J. KRAMER, WALTER RILEY, and T. T. BANNISTER, Duke University
Notes on the Myxophyceae of South Carolina with some additions to the flora of the state. E. C. COCKE and FREDERICK CORBIN, Wake Forest College
Differentiation in a callus culture of Sequoia sempervirens. ERNEST BALL, N. C. State College
Meiosis in normal and parthenogenetic Orobanche uniflora. H. W. JENSEN, Warren Wilson College
Notes on the distribution of North Carolina plants. W. B. FOX, R. K. GODFREY, N. C. State College, and H. L. BLOMQUIST, Duke University
x*The effects of irrigation with tap water on the inherent bioelectric surface potentials of Venus' fly-trap.* OTTO STUHLMAN, JR., U. N. C.
Genetic and environmental variation in Crotalaria. EARLENE ATCHISON, U. N. C.
x*Source of atmospheric salts.* S. G. BOYCE, N. C. State College

Application of a radioautographic technique to a study of carbon (C^{14}) fixation in Hydrangea *leaves.* D. E. MORELAND, N. C. State College, and W. M. DUGGER, JR., University of Maryland

Physiological response of axillary meristems of tobacco to mineral oil. D. B. ANDERSON, N. C. State College

x*The germination of sporangia of* Physoderma zeae-maydis *as influenced by environmental factors.* T. T. HEBERT and ARTHUR KELMAN, N. C. State College

Plant life on the reefs of the Carolinas. L. G. WILLIAMS, Furman University (Published in full in Jour. E. M. Sci. Soc. **67:** 133–161. 1951)

x*The effect of maleic hydrazide on some water relations of plants.* V. A. GREULACH, U. N. C.

Studies in the Cyperaceae of North Carolina. H. L. BLOMQUIST, Duke University

GEOLOGY SECTION

North Carolina pebbles. E. W. BERRY, Duke University

Ground water action on diorite in North Carolina. H. E. LEGRAND, U. S. Geological Survey

Post-Cretaceous faulting in Virginia. W. A. WHITE, U. N. C.

Massive topaz near Stem, North Carolina. S. D. BROADHURST, Department of Conservation and Development

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On certain recurrence formulas associated with the characteristic equation of a matrix. H. M. NAHIKIAN, N. C. State College

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VERNON KINROSS-WRIGHT, Durham Child Guidance Clinic and Duke School of Medicine
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Presidential Address: The fields and methods of applied experimental psychology. KEY L. BARKLEY, N. C. State College

WILDLIFE SECTION

Comparative growth rates of largemouth bass (Micropterus salmoides) *in North Carolina waters.* DUANE RAVER and J. H. CORNELL, Wildlife Resources Commission

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Great production in southern farm fish ponds. H. F. PRYTHERCH, Beaufort, N. C.

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x*Adaptive radiation in the snakes of the tribe Pseudoboini.* J. R. BAILEY, Duke University

Some studies on the growth and movement of hard clams (Venus mercenaria *L.*) A. F. CHESTNUT, Institute of Fisheries Research, Morehead City

Observations on Ophrydium versatile, *a little known colonial ciliate, from North Carolina.* E. C. COCKE, Wake Forest College

Bioelectric fields. J. L. EVERS, N. C. State College

Some observations on the life history of the glow worm, Phengodes latticollis *Lec.* B. B. FULTON, N. C. State College

x*Octonucleate and uninucleate structural units: Cytological contrasts in the larval and adult midguts of the parasitic wasp,* Habrobracon. D. S. GROSCH, N. C. State College

The faunal cycle in a small crepuscular cave. J. D. IVES, Carson-Newman

What's in a name. Z. P. METCALF, N. C. State College (Read by title only)

x*The preparation of a catalogue of the Nearctic Hymenoptera.* H. K. TOWNES, N. C. State College

x*Studies on the inheritance of rheumatoid arthritis in a Nash County pedigree.* MAURICE WHITTINGHILL, U. N. C., and EVELYN E. HENDRICKS, Nashville, N. C.

The separate effects of food and temperature on the length of larval period and size at metamorphosis of Ambystoma opacum. MARGARET M. STEWART, Woman's College, U. N. C.

Photoperiodism in hibernating mosquito larvae. C. E. JENNER, U. N. C.

NORTH CAROLINA SECTION OF AMERICAN CHEMICAL SOCIETY

The reproducibility of the manganese dioxide electrode with alkaline electrolytes. R. S. JOHNSON and W. C. VOSBURGH, Duke University

Recent advances in direct fluorination. A. R. GILBERT, MURRAY HAUPTSCHEIN, F. F. HOLUB, and L. A. BIGELOW, Duke University

The ion size parameter of hydrochloric acid in aqueous d-fructose solutions at 25°. H. D. CROCKFORD and A. A. SAKHNOVSKY, U. N. C.

Studies in the reduction of some carbon-nitrogen double bonds. J. A. MONTGOMERY and ARTHUR ROE, U. N. C.

Compounds of zirconium tetrachloride and hafnium tetrachloride with esters. W. S. HUMMERS, S. Y. TYREE, and S. YOLLES, U. N. C.

The reversible reaction of vanadate ions with acids. D. G. HILL and B. A. THUMM, Duke University

9-(p-hydroxyphenyl)-10-alkylphenanthrenes. C. K. BRADSHER and W. J. JACKSON, Duke University

Lambda-points of a symmetrical He^3-He^4 solution. J. C. MORROW III, U. N. C.

The reaction of acetone-1-C^{14} with sodium hypoiodite. E. L. ALBENESIUS and ARTHUR ROE, U. N. C.

Abstracts:

Cardiac Activity in Hypothermia. KENNETH E. PENROD.

That a fall in both heart rate and blood pressure occurs during progressive hypothermia in both cold and warm blooded animals is well established. Indeed all vital activity shows a progressive declination to a state approaching suspended animation at very low body temperature.

In the dog rendered hypothermic by immersion in an iced bath a large number of observations have been made on cardiac activity and its relation to other bodily functions. Although the heart rate and cardiac output decrease greatly at low temperatures, the bradycardia is not, as at normal temperatures, brought about through an essential lengthening of the diastolic period of the cycle, but in addition shows a vastly prolonged period of systole and isometric relaxation. This lengthening of the period of contraction, together with an increased blood viscosity and a profound leftward shift of the hemoglobin dissociation curve might be expected to decrease significantly the oxygen uptake of heart muscle to the point of serious impairment. But measurements of coronary blood dissociation show that the heart muscle is able to remove oxygen from the blood at an essentially normal rate even at extremely low partial pressures of oxygen at 20°C.

Further Observations on the Motile Cells of Actinoplanes. J. N. COUCH.

By observing the swimming spores of *Actinoplanes* under the dark field illuminated by a zirconium arc lamp, it has been found that the fast-moving spores do

not appear spherical but seem to be drawn out into a spiral with a maximum of about five complete turns. Spores moving less rapidly show fewer coils, and the ones swimming only moderately fast are practically round. The coiled spores may come to rest and instantly become rounded, and then may resume rapid motion apparently in the coiled shape. It is impossible at present to be sure if the coils are real or illusions. Each spore possesses a globule which shines brilliantly under the dark field. It could be that the spiral appearance is caused by the light reflected from this globule as the round spore moves spirally or merely rotates on its axis or zig-zags as it swims across the field of view. Several types of observations tend to show that the spirals are not illusions. After the spirals had been seen with the dark field they were visible with both the high power bright field and the phase microscope, but were much less distinct as spirals than under the dark field. Furthermore, by using very short exposures it has been possible to photograph the spirals. Whether the fast-swimming spores are actually drawn out in coiled fashion, or if this is some sort of optical illusion, the coils are very uniform in size, shape, and appearance when seen by the eye or camera. No known swimming fungal spore presents such a picture. It should be pointed out, however, that Pjiper has recently shown that the short rod-shaped organism of typhoid fever appears to be spiral when swimming rapidly. These and other observations previously published tend to show that *Actinoplanes* is more closely related to the bacteria than to the Phycomycetes.

The Effects of Irrigation with Tap Water on the Inherent Bioelectric Surface Potentials of Venus' Fly-Trap. OTTO STUHLMAN, JR.

That anesthesia, in the form of loss of response to electrical stimulation, can be produced by distilled water was shown by Osterhout and Hill (J. Gen. Physiol. **17:**, 87, 1933) to apply to their experiments on *Nitella* in which the cells lose their irritability when transferred from pond water to distilled water. Here anesthesia of the cell structure seems to be produced by a leaching process.

When tap water, containing air in solution, was used to irrigate the soil in which Venus' fly-trap (*Dionaea muscipula*) was growing, the static electrical potentials at various points on the surface of the trap, and its supporting spatulated petiole, rapidly changed with time. The direction of the change in bioelectrical potential due to the irrigation indicated that the current flowed from the proximal end of the petiole to the distal end of the trap. If the change is exclusively attributable to movement of inorganic ions then the logarithm of the E. M. F. could be a linear function of time. The present data show that the increase in negative E. M. F. is reducible to two logarithmic functions overlapping in time, so that probably two independent phenomena are involved.

Source of Atmospheric Salts. STEVE G. BOYCE.

Atmospheric salts have been of great interest to biologists and meteorologists since Pierre, in 1851, showed that rain water carried appreciable quantities of different salts. Meteorologists early recognized the importance of atmospheric salts and have shown that these tiny salt particles act as hygroscopic nuclei in

the atmospheric condensation process. Geologists have attributed the salinity of certain enclosed drainage basins, as Sambhar Lake, India, to the deposition of atmospheric salts. Salts from the atmosphere have been shown to be the source of soil iodine, which is essential in human nutrition. Recently it has been shown that the high concentration of atmospheric salts near the ocean is a factor in producing the shrub form and distribution of coastal plants. The sticky covering of salts on beach houses and the rapidity with which metal objects near the coast corrode have long been recognized as the result of salts carried by oceanic winds.

The general assumption has been that the sea was the primary source of these salts. However this has never been proved by discovery of the phenomenon by which these salts become air-borne. Köhler assumed that salt particles of one particular size are driven from the sea by winds. Clark proposes that they are raised from the ocean by vapor. Jacobs assumed that breaking waves, foam, and bursting bubbles produced aerosols by the mechanical dispersion of sea water.

After an extensive literature survey, the author was unable to find reference to experiments that demonstrated how oceanic salts become air-borne. Consequently, an investigation of the mechanics of this phenomenon was considered preliminary to an investigation of the effects of salt spray on coastal vegetation.

A salt sensitive paper, devised by the author, was used to show the primary process to be the action of bursting bubbles which eject tiny droplets of sea water into the atmosphere, the droplets then being transported by winds. When particles ejected by bursting bubbles of the ocean were collected on oiled glass slides it was found that they consisted of tiny concentrated droplets of salt water. The small diameter of the droplets and the high concentration of salts are due to rapid evaporation of the initial droplets to the same vapor pressure as the atmosphere. Laboratory measurements showed the droplets were ejected to sufficient heights to be caught in oceanic winds. Diameter measurements of the ejected droplets showed them to be small enough to be easily transported by normal winds according to Stokes' Law.

It is thus concluded that oceanic salts are ejected into the atmosphere, in tiny droplets of sea water, by the action of the ever present bursting bubbles of the ocean, and that these droplets are then transported inland by winds.

The Germination of Sporangia of Physoderma zeae-maydis *as influenced by environmental factors.* T. T. HEBERT and ARTHUR KELMAN.

In laboratory experiments temperature, pH, light intensity and light quality greatly influenced the germination of resting sporangia of *P. zeae-maydis*, the causal agent of the brown spot disease of corn. With a 72-hr. germination period the minimum and maximum temperatures for germination were 18° and 36°C., respectively, with the optimum between 27° and 28°C. When sporangial suspensions were exposed to light at 28°C. for 12 hrs. alternating with 12-hr. periods in darkness at temperatures ranging from 8° to 28°C., a high percentage of sporangia germinated throughout the range of temperatures. However, the sporangia germinated slowly at the lower dark-period temperatures. In potassium phos-

phate buffer solutions germination percentages were highest in the alkaline range. As the light intensity was increased from 0 to 12 foot-candles, germination percentages increased according to the logarithm of the intensity. Higher light intensities did not significantly increase germination. Under different wave lengths of light, high germination occurred in the blue, low in the green, and none in the yellow or red light.

The effect of Maleic Hydrazide on Some Water Relations of Plants. VICTOR A. GREULACH.

Moore (*Science* **112:** 52, 1950) reported that corn and beet plants treated with maleic hydrazide (MH) and growing in the field wilted severely, while the controls did not, and ascribed this to the small root systems of the treated plants. The writer noted that greenhouse tomato plants in pots wilted much less readily, if at all, when treated with MH than did the controls, and that the resistance to wilting increased with degree of growth inhibition, even though the shoot/root ratio also increased. These observations led to a study of the rates of transpiration of MH treated plants.

Measurements with cobalt chloride hygrometric paper indicated that treated bean plants were losing water vapor about $\frac{1}{3}$ as rapidly as the controls, while treated tomato plants were transpiring about $\frac{1}{4}$ as fast as the controls. The rates of transpiration of five plants each of treated and control beans and sunflowers over a 48 hour period were determined by the weighing method. The control plants of both species initially transpired more rapidly than the treated plants, both differences being statistically significant at the 5% level, but after the first day the transpiration rate per unit area of the controls decreased and was not significantly different from that of the MH plants. This was evidently due to a more rapid depletion of the soil water by the controls, both because of their greater leaf area and their higher initial rate of transpiration per unit area. The plants were not watered after the beginning of the experiment, and by the end all control sunflowers, but no other plants, were severely wilted.

The osmotic pressure of the leaves of the treated plants of both species, determined by the cryoscopic method, was higher than that of the controls, as might be expected from the accumulation of foods in the leaves of the plants treated with MH. The O. P. of the treated bean plants was 2.31 atmospheres higher than that of the controls, and that of the treated sunflowers 1.57 atmos. higher. Since Boone-Long (*Amer. Jour. Bot.* **28:** 333, 1941) found that an increase in O. P. of 2.2 atmos. could decrease transpiration as much as 36%, it appears that the observed differences in O. P. are sufficient to explain the differences in transpiration rates.

The stomates of sunflower controls closed within 2 hours after the plants were placed in the dark, but stomates of treated plants were still open after 4 hours in the dark, although they had closed after 12 hrs. This finding has no apparent relation to the transpiration differences being considered. In the beans the youngest leaves of the treated plants had only about 10% as many stomates per unit area as the controls, and development of guard cells had been halted in various

stages of their ontogeny. In the sunflower the treated plants had about half as many stomates per unit area on the youngest leaves as the controls, but the stomates were almost twice as large as on the controls. Since all these effects were limited to the young leaves it is doubtful if stomatal factors had any appreciable effect on transpiration differences. However, the treated leaves of both species were thicker and more compact than those of the controls, with thicker cell walls, and this may have affected transpiration as well as resistance to wilting.

The resistance of treated plants to wilting can be explained by: 1. The slower depletion of soil water by the treated plants, due to a lower rate of transpiration per unit area and a smaller leaf area. 2. A higher turgor pressure of the cells of treated plants, as a consequence of their higher O. P. 3. The greater mechanical support in the treated leaves. The more extensive root systems of the controls would be of little or no advantage to potted plants in delaying wilting, but would probably be advantageous under field conditions, as observed by Moore.

Both bean and tomato plants severely inhibited by MH had shoots which remained alive and turgid several weeks after the death of their roots, indicating that water absorption during this period was entirely passive.

Black Creek Cretaceous Deposits along the Cape Fear River, North Carolina. M. C. POWERS.

The Black Creek formation outcrops were studied along the Cape Fear River from 13 miles below Fayetteville, N. C., to 67 miles below Fayetteville. Laboratory investigation of selected samples included size analysis, heavy mineral analysis, estimation of roundness and sorting, and x-ray analysis.

The lower Black Creek, exclusive of the Snow Hill member, is composed of discontinuously bedded, interfingering mixtures of sand, silt, and clay. Many facies changes, sandstone lens, and soil horizons were observed in the field. These soil horizons represent diastems in the formation. No calcareous sediments were observed in the lower Black Creek, and it is void of marine fauna. *Dinosauria* fossils were present in the lower Black Creek. These observations point to a continental origin for this part of the formation. The Snow Hill member, of Stephenson, is a highly calcareous, evenly bedded, marine-fossiliferous sandstone. There is an unconformity between the Snow Hill and Black Creek. Because of this unconformity and the change from a marine to a non-marine sediment it is proposed that the Snow Hill member be known as the Snow Hill formation and the lower Black Creek be known as the Black Creek formation.

A Geophysical Survey over a Diabase Dyke near Chapel Hill. T. R. GOEDICKE.

To the south of Chapel Hill, the Triassic sandstone and the older slates are cut by several diabase dykes. These dykes strike in a north-westerly direction and the contacts are hidden under recent alluvium.

One of these dykes, on the property of Mason Farm, had previously been outlined by magnetic methods, and three electrical surveys were carried out over it in order to determine the value of electrical methods of geophysical prospecting in locating similar structures. The surveys were carried out along a series of

traverse lines crossing the dyke at intervals of 350 feet. Natural earth currents were measured and two resistivity surveys were made. Ground resistivity was measured to depths of 20 and 50 feet, using the Wenner method, and instruments constructed by the author.

There is little coincidence between known geological relations and the results of the self potential survey. This is apparently due to the fact that oxidation of the ferromagnesian minerals of the diabase proceeds too slowly for the development of potentials of any magnitude.

The best correlation between geophysical data and geology is found in the case of the resistivity survey measured to a depth of fifty feet. These results show an anomaly of 100 meter ohms over the location of the diabase dyke, and a slight decrease in resistivity on the uphill side of the dyke, due to water saturation of the Triassic sandstones. An area underlain by pre-Triassic slates is indicated by resistivity values in excess of 200 meter ohms, materially higher than resistivities found over sandstone areas.

The third electrical survey consisted of measurements of the ground resistivity to a depth of 20 feet from stations placed at 20-foot intervals. In this case, the dyke could not be correlated with any area of consistently greater resistivities. The area underlain by Triassic sandstones is marked by a series of parallel zones showing somewhat greater resistivity values than the surrounding areas. These zones are tentatively correlated with less porous or calcareous beds in the Triassic sediments. As in the case of the resistivity survey to a depth of fifty feet, the contact between the sandstones and slates is marked by a transition to much greater resistivity values.

It is concluded that resistivity measurements, to a depth of at least fifty feet, will reveal the presence of diabase dykes in sandstones, and locate contacts between the Triassic and older formations. It is proposed that this method could be used to determine the depth to the basement under the western part of the Durham Triassic basin.

The Relationship of Autonomic Reactivity and Selective Sensitization. WILLIAM K. BOARDMAN III.

In order to investigate the relationship between autonomic reactivity and the perceptual mechanism of selective sensitization, thirty-six stimulus words representing the six Spranger Value Categories were presented tachistoscopically to each of twenty-one male and twenty-one female subjects while connected in a potentiometric circuit. Each subject's recognition time of each word and his mean prerecognition resistance change for each word was determined. The Allport-Vernon *Study of Values* was administered for the purpose of indicating the relative values of the six Value Categories for each subject. Analyses of the relationship between *Study of Values* scores and recognition time, *Study of Values* scores and GSR, and recognition time and GSR were made in terms of chi-square values, rank-order correlations, and product-moment correlations.

The relation between *Study of Values* scores and recognition time was found to be reliably positive in nature for the men as a group, but varied as chance for the women. This finding of a direct relation between amount of recognition time

and amount of value category score contradicts the findings of earlier studies. No relation was evident between *Study of Values* scores and GSR. Recognition time and GSR were also found to vary together directly to a reliable degree with respect to the combined group of subjects, thus supporting previous contentions that selective sensitization and autonomic reactivity function together and are possibly causally related. The variations among individual records were so great, however, that the above mentioned relationships were demonstrable only with group data.

While these findings offer encouragement to further experimentation, they point up the necessity of refining both the techniques and the objective criteria employed in studying the effects of the meaning of a stimulus upon the perceptual processes. In addition, some modification in perceptual theory may be necessary to reconcile what appear at present to be contradictory findings.

The Derivation and Use of a Rating Scale to Evaluate the Level of Response in Thematic Apperception Test Protocols Given under Different Conditions of Administration. DOROTHY TERRY.

The Thematic Apperception Test (TAT) consists of a series of pictures, vague in outline, or ambiguous situations. The subject is asked to tell a story about each picture. In order to fulfill this assignment, the subject must impose his own interpretation upon these relatively unstructured pictures. The fundamental assumption of a projective test such as this is that the subject will, in making his interpretations, project his own personality into the stories, and reveal things about himself which he may be unwilling or unable to reveal in a more direct situation.

If we assume that the projective value of the TAT is a function of the subject's degree of involvement, then in order to investigate this relationship a method of quantifying the level of response is required. The degree of involvement, or level of response, was measured by an empirically derived rating scale.

The problem was first to establish a reliable scale and next to investigate differences in level of response to written and oral test administration, to the individual TAT cards, and among the individual subjects.

Forty women sophomore students in the elementary psychology course at the University of Wisconsin were given the 20 cards of the women's series. Half of the subjects wrote their stories and half gave them orally.

Each story was rated on a separate 10-centimeter line with no subdivisions, and with extremes ranging from a pure description of the picture, to detailed discussions of the motivation and analyses of the feelings of the story characters. A five-category scale was made, where each scale interval was described in terms of quotations from the stories within the interval. This scale was derived for use in the clinical situation. The correlation between ratings of 2 judges using this scale was .88.

For purposes of further analysis, a more finely subdivided scale with 20 intervals was used. These data were subjected to an analysis of variance. It was found that the written stories show a significantly lower average level of response than

do the oral stories. The cards show significant differences in average level of response elicited, and the subjects demonstrate consistent individual differences in level of response.

The Relationship between Visual Training, and Reading and Academic Improvement. H. C. OLSON, C. C. MITCHELL, and W. C. WESTBERG.

Problem: To determine the effect of a course in visual training upon the reading ability, academic performance, and personality inventory scores of college students low in academic standing.

Subjects: Sixty-five students, sophomores principally, in agriculture and education curricula at N. C. State College participated voluntarily.

Method: All subjects were tested before and after the experimental period on:

(1) Complete optometric examination
(2) Otis Test of Mental Ability (Higher Forms A and B—30 minutes)
(3) Iowa Silent Reading Test, New Edition (Form Am and Form Bm, Revised)
(4) Bernreuter Personality Inventory

The subjects were divided into four groups: (a) Visual Training, (b) Visual Training plus Counseling, (c) Counseling, and (d) Control. Treatments equated from the standpoint of visual training case type, Otis and Reading Test scores. Counseling of a vocational nature was included in the experimental design in an attempt to maintain the motivation of the subjects not receiving visual training.

Students reported for visual training for 45-minute sessions on alternate days three times per week over an eight-week period. Visual functions thought to be influenced by training with the Keystone apparatus used are speed and accuracy of fixation, fusion, size of the retinal area sensitive to form, and the speed and smooth action of gross eye movements.

Results: Using covariance, an "F" ratio significant beyond the 1% level was found for changes in reading rate. Differences between means, as determined by "t" ratios were significant at the 1% level for groups receiving visual training, but were not significant for those subjects in the Counseling and Control groups. No other "F" values were statistically significant at the 5% level. However, non-significant trends toward improvement in favor of visual training are indicated for reading comprehension and academic performance in the term following the experiment. Bernreuter stanine scores show trends toward improvement in favor of counseling.

Conclusion: Sophomore college students low in academic performance who undertook a course of visual training show gains in reading rate significant at the 1% level. Non-significant gains also are shown for reading comprehension and grade-point averages. Students in the counseling treatments demonstrate favorable non-significant trends toward improvement in Personality Inventory scores.

Adaptive Radiation in the Snakes of the Tribe Pseudoboini. J. R. BAILEY.

The Pseudoboini is a natural supergeneric group composed of eight genera of neotropical nocturnal snakes: *Pseudoboa, Clelia, Rachidelus, Phimophis, Drepa-*

noides, Oxyrhopus, Siphlophis and *Tripanurgos*. Of these *Pseudoboa, Clelia* and *Oxyrhopus* are good-sized genera with considerable morphological variation, but the others contain from 1 to 4 species, and each genus represents a distinct morphological type.

Within this restricted natural group adaptive radiation as an evolutionary principle is clearly evident as reflected in the habits and structure representing the several evolutionary lines. The ancestral or primitive morphological and habitudinal type is postulated. The three largest genera have departed little from this type and represent more or less generalized terrestrial forms, although in each genus there are species of reduced size and scutellation and more or less fossorial habit. *Clelia* also includes very large species which are specialized to feed on large poisonous snakes. The small genera represent three separate invasions of the arboreal habitat and two distinct developments of fossorial types. Structural adaptations to these modes of life are pointed out.

Octonucleate and Uninucleate Structural Units: Cytological Contrasts in the Larval and Adult Midguts of the Parasitic Wasp, Habrobracon. D. S. GROSCH.

Unusual cytological features discovered in the spinning gland of the braconid known in genetic literature as *Habrobracon juglandis* (Ashmead) were presented to the North Carolina Academy of Science in 1950. These included the formation of quadrinucleate structural units through amitotic division of "macronuclei". The present report concerns another larval tissue and introduces a structural unit containing a still higher number of nuclei. By the last instar, the larval midgut is composed chiefly of huge flat epithelial plates each containing 8 nuclei! These structural units have been derived by amitotic nuclear divisions from units with fewer nuclei such as the quadrinucleates, which are common in first and second instars, and the uninucleates and binucleates of the early first instar. Endomitotic sequences seem to occur for the nuclear contents preceding amitotic division and during subsequent increase in size of the nuclei accompanying growth of the unit. During metamorphosis, when the larval midgut is histolyzed, all nuclei of a specific unit are in a similar stage of degeneration. This aspect, especially demonstrable with the Feulgen technique, furnishes evidence that the octonucleate structure functions as a physiological unit. Around the greatly contracted remnant of the degenerating larval midgut, the adult midgut is formed of uninucleate cells. This imaginal organ is no larger than one-third the size of the huge larval sac which once occupied almost 90% of the developing organism (Grosch, Jour. Elisha Mitch. Sci. Soc. **65:** 61–70).

An explanation of why multinucleate situations have not been previously described for parasitic wasps lies, perhaps, in the fact that for many years sectioning methods have been emphasized, whereas the present observations were made on whole mount preparations using histochemical techniques. Demonstration of multinucleate structure elucidates observations by both the present author (Jour. Morph. 1950) and earlier investigators (Genieys, Ann. Ent. Soc. Am. 1925), who noted an apparent infrequency of cell walls in sectioned braconid tissues. Furthermore, these discoveries form part of a conception which reveals

similarities between cytological features of ciliated protozoa, long considered unique, and events in a highly evolved member of the metazoa.

The Preparation of a Catalogue of the Nearctic Hymenoptera. HENRY TOWNES.

The latest catalogues or comprehensive treatments of any kind of the Nearctic Hymenoptera are Cresson's Catalogue and generic synopsis, published in 1888, and Dala Tore's "Catalogus Hymenopterum", a world catalogue published in installments at the turn of the century. These two publications have been foundations for a lot of work, but are now out of date and inadequate. After discussing the matter with a number of students of the Hymenoptera, it was decided that with a joint effort a new catalogue could be produced that would not only codify the existing published information, but would record a myriad of facts regarding synonymy, classification, and biology that were in the possession of specialists but which had never been published. The result would be a guide to this large and complex group of animals that would start students on further work at a much higher level than otherwise possible, and would serve as a comprehensive reference manual on the bibliography, nomenclature, distribution, and biology of the Hymenoptera for all interested zoologists.

An outline of the proposed scope and nature of the catalogue and a sample portion of manuscript to show bibliographic style were prepared and sent to the specialists who might assist. Messrs. C. F. W. Muesebeck and K. V. Krombein of the U. S. Department of Agriculture joined me in this project at that time and we three have served as editors. There was good response to our proposal and early in 1947 work on the manuscript was begun by about twenty workers scattered all over the United States, and by one important contributor in Canada. In the case of some groups there was no qualified specialist, or the one person who seemed qualified did not care to join the project. In these cases one of the three editors took the job. There was, of course, a lot of correspondence with our contributors about style, content, and nomenclature and a little prodding to be done. The manuscript was finally brought together late in 1949, retyped and edited early in 1950, and has just been published. There are about 1,500 pages (in small type) in the catalogue, treating about 15,000 species known from America north of Mexico.

Studies on the Inheritance of Rheumatoid Arthritis in a Nash County Pedigree. MAURICE WHITTINGHILL and EVELYN E. HENDRICKS.

In a search for the mode of inheritance of rheumatoid arthritis a pedigree of over 1000 persons has been compiled by the junior author. By means of medical diagnoses cases of rheumatoid arthritis and of spondylitis have been distinguished from osteoarthritis, Heberden's nodes and other unrelated conditions. Most of the cases of permanent joint damage due to rheumatoid arthritis are concentrated in one part of the pedigree embracing 110 persons in the direct line of descent from a common ancestor. The 4 or 5 cases of spondylitis in males may also be traced to the same progenitor. Spondylitis is known to affect males 9 times as often as females, and inheritance as a dominant with low penetrance has been

suggested. From our figures a penetrance value of 18% for spondylitis in male heterozygotes has been computed.

Rheumatoid arthritis seems to follow a similar dominant type of inheritance but affecting both sexes about equally. We have ruled out recessive inheritance after study of ten cousin marriages in the pedigree. On the dominant hypothesis, 9 cases in sibships totalling 48, or 10 out of 56, give penetrance values of 31 per cent and 29 per cent, respectively, for crippling cases after correction for sibship sizes. These figures compare satisfactorily with figures derivable from studies by Gunther Brandt and Frederick A. Weihe, 1943, on monozygotic and dizygotic twins, at least one of which had polyarthritis rheumatika. From material which they saw, and from other classifiable pairs which they found in the literature, one may compute penetrance values of 38%, 28%, 23% and 18%.

Our study will continue with the purposes of refining the penetrance figures above, of studying cases which are not crippling, of investigating the apparent relationship between spondylitis and rheumatoid arthritis encountered here, and of following up certain other inherited muscular and skeletal conditions.

REINARD HARKEMA, *Secretary*

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

1950–1951

482ND MEETING, OCTOBER 10, 1950

A. V. MASKET (Physics): *The Operation of Uranium Chain Reactors.*

In order to bring a nuclear chain reactor to criticality, great care must be exercised, in particular in connection with reactors of small volume which can rise very rapidly in activity beyond the self-sustaining condition. In order to estimate how close the multiplication factor of the reactor may be to unity (criticality condition), the convenient procedure is to introduce into the reactor an artificial source of neutrons whose activity is q neutrons/sec. If each neutron, so introduced, gives rise to the production of k neutrons on the average after all events it may suffer, then the total number of neutrons produced per neutron is

$$K = k + k^2 + k^3 + \cdots = \frac{k}{1-k}, \qquad k < 1$$

In the steady state, the reactor will be producing

$$Q = K \cdot q = \frac{qk}{1-k} \quad \text{neut/sec.}$$

A plot of active mass vs $1/Q$ will yield, on extrapolation, a measure of the nearness to criticality.

JAMES C. ANDREWS (Biological Chemistry and Nutrition): *Studies on Phytin.*

Phytin is the mixed calcium-magnesium salt of the hexaphosphoric acid ester of inositol. From commercial phytin a recrystallized hexabarium phytate was prepared and analyzed. From this barium salt there were prepared both the sodium salt and free phytic acid.

The titration curve of free phytic acid with alkali was determined by means of the glass electrode and the approximate positions of each of the two main groups of six ionizable hydrogen atoms on the pK scale were determined. The first six moles of alkali added per mole of phytic acid produced a fairly vertical curve lying between pH 2 and pH 5. This was succeeded by a comparatively flat portion which soon rose to include the second set of six inflections, somewhat more widely spaced than the first set of six. At pH 11 titration of all twelve equivalents was complete.

A solution of sodium phytate was prepared from the barium salt and used as a substrate to test for phytase activity in a number of microorganisms from air, soil, and the intestinal tract. From twenty-four microorganisms tested, eight showed some ability to hydrolyze sodium phytate. The others gave negative results. However, those which gave positive results were organisms present too

far down in the intestinal tract to make possible any absorption of the resulting inorganic phosphate. For this reason the hydrolysis of phytin in the intestinal tract due to microbic action is apparently not practicable with any of the organisms tested.

483RD MEETING, NOVEMBER 14, 1950

FRED SEMENIUK and W. D. CASH (Pharmacy): *Instability Characteristics of 1-Haloalkyl Ester.*

C. R. WALTER, JR. (Chemistry:) *Quinonimides: The Diels-Alder Reaction.*

It has been found that the alkylsulfonyl and arylsulfonyl derivatives of p-phenylenediamine can be oxidized to the corresponding p-quinonedisulfonimides which are stable. These compounds exhibit many of the reactions of quinone.

When p-quinone dibenzenesulfonimide is allowed to react with butadiene, a normal addition apparently first takes place which is immediately followed by aromatization to form 5,8-dihydronaphthalene-1,4-dibenzenesulfonamide. p-Quinone dimethanesulfonimide reacts in a similar fashion. The reaction between diphenylbutadiene and p-quinone dibenzenesulfonimide occurs with the formation of the normal adduct, and it can be rearranged to 6,7-diphenylnaphthalene-1,4-dibenzenesulfonamide by warming with hydrobromic acid. The use of cyclopentadiene in the same reaction gives the di-adduct, two molecules of the diene adding to one of the dienophile.

The Diels-Alder reaction apparently does not take place when anthracene is allowed to react with p-quinone dibenzenesulfonimide. Instead a 1,4-addition to the quinone diimide occurs followed by aromatization. The attachment is assumed to be in the 9-position, since by the action of bromine a dibromo adduct does not form but instead a monobromo substitution product, probably the 10-bromo compound.

484TH MEETING, DECEMBER 12, 1950

HERBERT E. ROBBINS (Mathematical Statistics): *A New Approach to the Theory of Testing a Statistical Hypothesis.*

C. E. JENNER (Zoology): *Can Snails Tell Time?*

485TH MEETING, JANUARY 9, 1951

G. R. MACCARTHY (Geology): *A Scientist in the Arctic.*

486TH MEETING, FEBRUARY 13, 1951

S. B. KNIGHT (Chemistry): *Flame Photometry.*

WARFIELD GARSON (Public Health): *Recent Advances in Laboratory Diagnosis of Syphilis.*

Syphilis, although still one of the most important public health problems in our society, appears to be decreasing in our population. However, in that population there are a relatively constant number of people who have false positive

reactions to the present standard test for syphilis. These tests measure reagin which is an antibody in the serum unrelated directly to the syphilitic infection. As the number of cases of syphilis in the population decreases and the number of people reacting with a false positive to the standard tests remains relatively constant, we obtain an increasing proportion of people reacting positively to these tests who do not have syphilis as compared to those who do, thus eventually limiting the usefulness of the present tests considerably.

It is imperative, therefore, that the laboratory attempt to find new means to aid in the diagnosis of syphilis and to base these new techniques on some biological phenomenon other than the presence of a nonspecific antibody such as reagin in the serum.

The more recent improvements and modifications of the reagin tests were discussed in terms of the limits of their contribution to the problem. These were the development, use and results with the purified cardiolipin-lecithin-cholesterol antigen, and the Filter Paper Microscopic (FPM) test for syphilis.

The two newest developments predicated upon biological phenomena other than the testing for reagin were presented. The techniques considered were the Euglobulin Inhibition Test and the Treponemal Immobilization Test (TPI). It was suggested that this latter test represents the most specific test for the treponematoses yet devised.

The discussion was closed with a consideration of future problems in this field which included the *in vitro* growth and reproduction of the pathogenic *Treponema pallidum*—a problem yet unsolved after half a century.

487TH MEETING, MARCH 13, 1951

C. T. KAYLOR (Anatomy): *A Radioautographic Study of the Localization of Carrier-free Radioberyllium in the Rat.*

EARLENE ATCHISON (Botany): *Geography and Variation in Crotalaria.*

488TH MEETING, APRIL 10, 1951

F. BURTON JONES (Mathematics): *Topology as I Have Encountered It in Everyday Life.*

JOHN E. LARSH, JR. (Parasitology): *Host-Parasite Relationships in Cestode Infections, with Emphasis on Host Resistance.*

At this meeting Dr. Costello presented the need for an increase in the annual appropriation to the Journal of the Society. He reported that the Executive Committee recommended that those members who wished to receive the Journal should pay $2.00, which would include dues and subscription; that associate members should pay $1.00 for the subscription to the Journal, in addition to their membership fee; that the present contribution of $50.00 from the Society be continued and that the extra dollar from each member wishing a subscription go to the Journal in addition.

After a brief discussion Dr., Ferguson moved that the regular annual dues of active members be $2.00; that the Society continue its annual contribution of

$50.00 plus $1.00 of each member's dues to the Journal; and that associate members who wish to receive the Journal pay $1.00 for a subscription, in addition to associate membership fee of 25 cents.

This motion was unanimously passed by the Society.

After some further discussion it was also moved and passed that the number of members of the editorial board should be left to the discretion of the Editor-in-Chief.

489TH MEETING, MAY 8, 1951

TALBOT ALBERT CHUBB (Physics): *A Study of the Philips Gage Discharge.*

W. J. DANIEL (Psychology): *Cooperative Problem Solving in Rats.*

At the beginning of this program Dr. Costello presented a certificate and a check for $50.00 to Mr. Talbot Albert Chubb, as winner of the William Chambers Coker Award in Science for the year 1950–51.

Dr. J. C. Andrews, chairman of the Nominating Committee, presented the following slate of officers for the year 1951–52:

President—H. D. CROCKFORD
Vice-President—W. R. STRAUGHN
Secretary-Treasurer (2-year period)—W. A. BOWERS
Editor-in-Chief of the Journal (5-year period)—J. N. COUCH

The above officers were unanimously elected.

Dr. R. E. Coker and Dr. W. deB. MacNider were elected honorary members of the Society.

ALBERT E. RADFORD, *Sec.-Treas.*

DARTERS OF THE GENUS HOLOLEPIS FROM SOME NATURAL LAKES OF NORTH CAROLINA

By Joseph R. Bailey[1] and David G. Frey[2]

Plates 6–8

Knowledge of the fishes of the natural lakes of North Carolina has been very meager until recently. Frey (1951b) summarizes previous contributions to the knowledge of the ichthyofaunas of lakes Jones, Salters, Singletary, White, Black, and Waccamaw, and discusses the fishes collected during a biological survey of these lakes made for the North Carolina Wildlife Resources Commission in the summer of 1947. The present paper deals only with the darters of the genus *Hololepis* collected during this survey, supplemented by a collection from Ellis Lake obtained by Frey in the summer of 1950. These 7 lakes are the only North Carolina lakes for which material is available.

At the time Hubbs and Cannon (1935) published their revision of *Hololepis*, the only records for the genus from the natural lakes of North Carolina were those of Smith (1907) and Brimley (1909) for *Hololepis serrifer* from Ellis Lake, based on the same specimens. The species was reported as *Copelandellus* (or *Copelandella*) *quiescens*. In addition there are doubtful records of *H. thermophilus* from Lake Mattamuskeet. In a semi-popular article on the results of the fisheries survey mentioned above, Hueske (1948) listed all the darters collected as *Boleosoma* sp., including the *Hololepis* material of this paper, as well as *Boleosoma perlongum* of Lake Waccamaw. *Hololepis* was collected in all the lakes investigated except Black Lake, where the form apparently has not become established.

The materials reported in this paper include rather extensive series of *Hololepis thermophilus* from Jones, Salters, Singletary, and White lakes, and smaller series from lakes Ellis and Waccamaw. In addition there is a small series of *Hololepis serrifer* from Ellis Lake. This is the only North Carolina lake in which the two species mentioned have been found in the same water, and in fact both species apparently occur in identical habitat because they were taken simultaneously with a dip net in weed beds over a sandy bottom. Hubbs and Cannon (l.c.: 67) point out that *thermophilus* and *serrifer* are the only two species of the genus which have ever been taken in the same collection.

The observed variation in *Hololepis thermophilus* is so extended by the present study that it seems best to divide the species into two subspecies:

Hololepis thermophilus thermophilus Hubbs and Cannon, from lakes White Waccamaw, and Ellis, and localities in original description.

Hololepis thermophilus oligoporus, new subspecies, from lakes Jones, Salters, and Singletary.

[1] Department of Zoology, Duke University.

[2] Department of Zoology, Indiana University. This paper is in part Contribution No. 469 from the Department of Zoology, Indiana University.

THE LAKES AND THEIR GENERAL ECOLOGY

Lakes Jones, Salters, Singletary, White, and Waccamaw, known as "bay" lakes because they occupy basins similar to thousands of other "bays" no longer containing lakes, have already been reported on in detail by Frey (1949). The five lakes, including Ellis, of the Craven-Jones region of the North Carolina Coastal Plain, although undoubtedly different in origin, are quite similar in general chemistry and morphometry. The discussion of the chemistry of the latter lakes is based on a series of samples collected from Ellis, Catfish, Little, and Great lakes on February 5, 1949, and the discussion of the morphometry is based on this visit plus two additional ones.

All these lakes are shallow, not exceeding 12 feet in depth. The maximum depth of Ellis Lake is scarcely more than 4 feet. Waccamaw at 7.6 ft. and White at 7.5 ft. have the greatest average depths. The shoreward portions of these lakes, except for the northwest end of the Bladen County bay lakes, is sand, with soft organic sediments in the center. Brown (1911) reported a somewhat similar soft central portion for Ellis Lake, and this was observed.

All the lakes except White Lake have a detectable brownish color. The color of White Lake is less than 10 ppm. on the platinum-cobalt scale, that of Singleary and Waccamaw about 165 ppm., of Ellis 210 ppm. (on February 2, 1949), and of Jones and Salters approximately 300 ppm. One effect of this color, of course, is to reduce greatly the extent of light penetration. Except for Waccamaw, which has a neutral chemical reaction and a somewhat higher calcium content, the lakes have a pH between 4.3 and 5.0, with almost negligible quantities of chemically active substances.

Lakes Jones and Salters have virtually no aquatic vegetation in them except for *Sphagnum* and *Fontinalis* attached to cypress roots. Singletary has a somewhat greater amount of vegetation, especially in an artificial channel at the outlet. White Lake has low and sparse vegetation covering the entire bottom below the level of wave action, with open beds of *Panicum* along the sandy shores. Waccamaw has considerably more vegetation, both as to number of species and quantity. Ellis Lake in summer is almost completely choked with a variety of rooted aquatics, first described by Brown (1911). This exuberance is made possible in spite of the considerable color of the water by the very shallow depth. In the late 1800's Ellis Lake was drained and used for rice production, so that an altered edaphic factor may also be contributing to the success of the vegetation.

In the bay lakes reported on by Frey (1949), Lake Waccamaw is by far the most productive, followed in order by White, Singletary, Jones, and Salters. Ellis Lake has large fish populations, and might be even more productive per unit area (or volume?) than Waccamaw. Certainly no other lake of this present group supports breeding colonies of cormorants, little blue herons, black-crowned night herons and other piscivorous birds, as well as otters. A variety of ardeids can be observed intently fishing in shallow water whenever the lake is visited in warm weather.

As to the drainage systems involved, Jones, Salters, Singletary, and White lakes are all in the Cape Fear drainage, Lake Waccamaw heads the Waccamaw

River to the south, and Ellis Lake drains into the lower portion of the Neuse River estuary to the north.

The specimens of *Hololepis* reported on in this paper were observed at all depths of water and over all types of bottom. In Jones Lake they were collected along the sand shore almost up to the edge of the water, and in Waccamaw they were collected almost one-half mile from shore in water 8 feet deep. Specimens were taken over both sandy and soft organic bottom. It is thus believed that the species *Hololepis thermophilus* is quite generally distributed over the bottoms of these lakes. Obviously they are important in the food chains in the lakes, and Hueske (1948) has reported that they are eaten extensively by largemouth bass in White Lake.

Hololepis thermophilus thermophilus Hubbs and Cannon

Material examined

White Lake: 34 specimens, taken mostly in *Panicum* bed or among cypress roots, on soft or sand bottom, 1–4′ deep.

Waccamaw Lake: 8 specimens, one in main inlet 75 yards upstream, 10–15′ deep; others in *Panicum* beds, among cypress roots, or in open lake, 2½–8′ deep, near shore to 750 yards offshore.

Ellis Lake: 11 specimens, among weeds in shallow water.

A total of more than 250 specimens of this species were collected from White Lake and approximately 50 specimens from Waccamaw, but most of this material was unavailable for study. Data on the meristic characters of the specimens examined are presented in Tables 1 to 4. Comparison with the new subspecies and discussion of variation, relationships, and distribution are presented in later portions of the paper.

Hololepis thermophilus oligoporus, new subspecies

The populations in Jones, Salters, and Singletary lakes differ significantly from typical *thermophilus* in having fewer pores in the lateral line and on the head.

Material examined

Jones Lake: 96 specimens, among cypress roots and in *Xyris* bed, sand bottom, 1½–3′ deep.

Salters Lake: 50 specimens.

Singletary Lake: 33 specimens, in outlet channel and along lake shore, among cypress roots or not, on soft or sand bottom, 2–4′ deep.

HOLOTYPE: An adult male 25.7 mm. in standard length, from Jones Lake collected September 10, 1947, by the Lake Survey party.[3] Paratypes are those additional specimens listed under material examined. In the following description data in parentheses refer to the holotype.

DIAGNOSIS: Differs from the typical subspecies in having fewer pored scales, more unpored scales, and a lower ratio of pored to unpored scales in the lateral line row; pores in the infra-orbital canal usually 1 + 3 instead of 2 + 3; total number

[3] The holotype and series from each lake will be deposited in the University of Michigan Museum of Zoology.

of scales in the lateral line row slightly more; maximum size apparently slightly smaller; coronal pore reduced in size, but usually present.

BODY: Very elongate, moderately compressed.

HEAD: Muzzle blunt; mouth subterminal, slightly oblique; lower jaw included. Preopercle smooth, rarely with a few serrations on the vertical limb (serrations 2–3). Distance from gill membranes to tip of snout greater than half the length of head.

GENITAL PAPILLA: In mature female conical without basal enlargements, opening by a slit in the ventral surface, overlapping origin of anal by nearly half its length, turgid, held out at an angle to the body. In mature male small, conical, lying in a groove and extending only about half way from its origin to the origin of the anal.

Breeding tubercles not apparent.

FIN RAYS: Dorsal, VII to X–7 to 11 (IX–8); anal II–6 to 9 (II–7).

SCALE ROWS: Two to 4, usually 3–45 to 61–7 to 9 (3–51–8).

LATERAL LINE: Extremely short, occasionally lacking entirely; pored scales 0–15 (8 on left, 6 on right), average 6.8; unpored scales in same row 35–59 (43), average 44. Ratio of pored to unpored scales 0–.38 (.19), usually .03–.23). Scales above rudimentary extension of lateral line at origin of second dorsal 2–4, usually 4 (4).

SQUAMATION: Breast usually with embedded cycloid scales (embedded), rarely naked or with exposed cycloid scales, but one noted with exposed ctenoid scales. Nape scales well embedded to exposed in Jones Lake (exposed), exposed and ctenoid in Singletary Lake, or embedded to wanting in Salters Lake. Embedded scales most frequent in paravertebral rows. Cheek scales exposed and ctenoid, rarely embedded ventrally. Opercles covered with ctenoid scales. Parietal region scaleless dorsally; its posterolateral edges above the lateral canal and in front of the supratemporal canal with 3–5 horizontal rows of scales, usually the two lowermost reaching the supraorbital canal and only 1 or 2 often embedded cycloid scales representing the uppermost row (4 rows, 2 reaching supraorbital canal, 2 scales in uppermost row and scaleless for median half of area above lateral canals). Interorbital with 1–4 (3), usually 2–3 partially embedded ctenoid scales in a single, rarely double, row.

HEAD PORES: Interorbital pores constantly lacking. Infraorbital canal interrupted, usually with 1 pore, occasionally 2, behind the break, and 3 pores above the upper jaw (1 + 3). Operculomandibular pores 9 (9), occasionally 10. Supratemporal canal most frequently complete (complete) with a single median pore, but very commonly interrupted (usually so in Singletary Lake), with paired pores separated by the width of 1 to several pore diameters. Coronal pore much reduced in size and frequently lacking (lacking).

COLORATION: Sides with a row of 8–13 (9) dark blotches, the first above the tip of the opercle, the others along the midline of the sides. Smaller, lighter secondary markings may alternate with the blotches. Back with 7–10 (8) dark saddles, 1 just behind the occiput, 1 before the first dorsal, 2 below the first dorsal, 1 or 2 below second dorsal and 3 on caudal peduncle. Upper sides between lateral and

dorsal rows of blotches a monotone usually about half as dark as blotches, but with much individual variation. Lateral line marked by a narrow light line, often obscure. Base of caudal with 3 dark spots in a vertical row, the median one usually most intense. A vertical dark bar behind base of pectoral. Upper cheek and opercle mostly dark, lower cheek and opercle light with clusters of dark chromatophores. Lower jaw, throat, and breast light, frequently with clusters of chromatophores. Four short dark lines or spots radiating from eye, the suborbital largest. Parietal region dark, nape light between first 2 dorsal saddles.

Female with belly, lower sides, and ventral surface of caudal peduncle light, usually with chromatophores much scattered or clustered into spots. Male frequently with a regular dark suffusion of chromatophores over this area (probably breeding coloration), or this area evenly but sparsely speckled (probably non-breeding coloration). Both types of male coloration and intermediate conditions may be present in the same collections and perhaps reflect differential retention of breeding coloration into the summer.

Spinous dorsal of the male with a submarginal band of dark chromatophores on the membrane; below this an area which is clear or with few chromatophores; basal half to two-thirds of fin with large irregular chromatophores, sometimes so concentrated as to appear as dark vertical bars on the membrane. Soft dorsal similarly but less intensely marked. Anal similar but lacking the submarginal chromatophores. Pelvics usually with some scattered basal chromatophores. Pectorals usually clear. Intensity of fin coloration proportional to degree of belly pigmentation and highly variable in same collections. All vertical fins more or less barred on the rays (anal very slightly so), but this largely obscured except on the caudal in more highly colored individuals.

Fins of female show patterns similar to those of male but with very much reduced pigmentation on the membranes, at times completely lacking. The barring of the dorsal fins by contrast stands out much more boldly.

VARIATIONS

The variations in the more important meristic characters of *Hololepis thermophilus* are tabulated for the several populations in Tables 1 to 4. Included for comparison are the data for most of these characters as tabulated for *thermophilus* by Hubbs and Cannon (l.c., Table II). There is no indication that their material included specimens of *oligoporus*. Table 4 summarizes the more important subspecific characters, indicating the percentage of distinction found for each character. Careful inspection of the tables will reveal that each population shows characteristics of its own. In the diagnostic characters of the lateral-line row and the infraorbital pore series, however, there is a strongly marked dichotomy which is reflected in the two subspecies we recognize. In the characteristics of the lateral-line row the population of Singletary Lake most closely approaches typical *thermophilus*. In the arrangement of infraorbital pores, however, it agrees perfectly with the populations from Salters and Jones Lakes. Conversely, in the latter character the population from Lake Ellis, geographically far removed from the other lakes under consideration, shows a marked approach to the reduced

TABLE 1

Frequency distribution of numbers of scales in the lateral line row in the two subspecies of Hololepis thermophilus

NUMBER OF SCALES IN LATERAL LINE ROW	*t. oligoporus*									*t. thermophilus*													TOTAL *t. oligoporus*			TOTAL *t. thermophilus*		
	Jones			Salters			Singletary			White			Waccamaw			Ellis			*thermophilus* from Hubbs and Cannon (1935: Table II)											
	P	UP	T	P	UP	T	P	UP	T	P	UP	T	P	UP	T	P	UP	T	P	UP	T	P	UP	T	P	UP	T
0	8																						8					
1	9			2			1																12					
2	5			1			1																7					
3	5			2			1																8					
4	10			1			–																11					
5	6			1			–																7					
6	1			1			2																4					
7	9			2			2																13					
8	17			6			1																24					
9	11			4			3																18					
10	5			1			4			1									1				10			2		
11	4			–			6			–									2				10			2		
12	3			–			5			2			2						3				8			7		
13	1			–			2			2			–			4			–				3			6		
14	1			1			1			3			–			–			3				3			6		
15							1			3			–			3			6				1			12		
16										4			2			1			4							11		
17										3			2			1			–							6		
18										–						1			–							1		
19										–						1			1							2		
20										2									1							3		
21										1																1		
22																												
23																												
24											1																1	
25											–																–	
26											–									1							1	
27											–						1			–							1	
28											1						–			–							1	
29											1						–			2							3	
30											–						1			–							1	
31											–			1			2			2							5	

32				1	–	3	2		6
33				5	–	–	3		8
34				1	–	1	5		7
35	1			–	1	1	3	2	5
36	–		1	1	2	2	2	1	11
37	1	2	1	2	–	2	2	4	5
38	–	1	2	2	2			3	4
39	4	2	5	7	–			13	–
40	9	5	1	3	–			17	–
41	6	1	–	2	–			9	–
42	8	1	2	2	–			11	–
43	9	–	3	3	–		1	12	1
44	8	2	2	2	–		2	12	3
45	8	1	3	2	1		3	13	2	4
46	5	–	1	1	–	1	1	6	3	6
47	10	3	2	–	1	1	3	12	5	11
48	5	9	3	2	1	2	3	8	17	11
49	3	11	6	3	4	4	5	5	17	8
50	4	15	3	7	4	4	3	5	25	2
51	5	21	4	4	4	1	1	5	29	6
52	7	11	–	4	3	3	–	7	15	2
53	1	11	1	3	2	2	1	1	15	–
54	–	6	–	2	–	–	1	–	8	1
55	–	4	1	1	–	–		–	6	–
56	–	1		2	–	–		–	3	1
57	–	–			–	1		–	–	
58	–	1						–	1	
59	1	–						1	–	
60		–							–	
61		1							1	
Mean P	5.95		6.68	9.60	15.38	15.00	15.18	14.43	6.80	14.97
Mean UP	45.13		42.45	41.47	34.57	35.67	32.36	33.18	43.98	33.77
Mean T	51.07		49.14	51.07	49.95	50.67	47.55	47.45	50.78	48.67

P column = pored scales: UP column = unpored scales; T column = total scales.

pore series found in *oligoporus*, but its lateral-line row characteristics are those of typical *thermophilus* as recorded by Hubbs and Cannon (l.c.) and as found in the White and Waccamaw populations.

TABLE 2

Frequency distribution of sensory pore characters in the two subspecies of Hololepis thermophilus

		l. oligoporus			*l. thermophilus*				TOTAL *l. oligoporus*	TOTAL *l. thermophilus*
		Jones	Salters	Singletary	White	Waccamaw	Ellis	Hubbs and Cannon (1935: Table II)		
Ratio of pored to unpored scales	.00	8							8	
	.03	14	3	2					19	
	.08	16	3	1					20	
	.13	8	2	2					12	
	.18	28	8	6					42	
	.23	13	5	5	1				23	1
	.28	3	–	6	–			2	9	2
	.33	3	–	5	3	2		4	8	9
	.38	2	1	3	4	–	3	1	6	8
	.43				6	2	2	8		18
	.48				1	1	3	4		9
	.53				3	1	1	–		5
	.58				–		1	–		1
	.63				–		–	–		–
	.68				1		1	1		3
	.73				1			1		2
	.78				–					–
	.83				–					–
	.88				1					1
Infraorbital pores	0-3				2					2
	1-3	62	56	61	3	2	10		179	15
	2-3	4	4	3	26	12	12	24	11	74
	3-3				3	2				5
Operculomandibular pores	7				2					2
	8				3			1		4
	9	57	60	61	15	15	22	11	178	63
	10	7		3	1	1			10	2
Median supratemporal pores	1	25	17	13	15	7	10	19	55	51
	2	6	13	19	4	1	1		38	6
Coronal pore	0	7	6	10	14	2	8		23	24
	1	16	24	20	4	6	3		60	13

In maximum size (Table 4) the differences are slight and of questionable significance. All three populations of *oligoporus* fail to attain the size found in the

other lakes, which are much more productive, and might be expected to produce larger fish.

TABLE 3

Frequency distribution of certain meristic characters in the two subspecies of Hololepis thermophilus

		t. oligoporus			*t. thermophilus*				TOTAL *t. oligoporus*	TOTAL *t. thermophilus*
		Jones	Salters	Singletary	White	Waccamaw	Ellis	Hubbs and Cannon (1935: Table II)		
Dorsal spines	VII		1						1	
	VIII	6	16	5	1	3			27	4
	IX	21	17	23	7	4	7	6	61	24
	X	6	3	4	11		3	10	13	24
	XI				2		1			3
Dorsal soft rays	7	1							1	
	8	1	1	4	1	2		2	6	5
	9	20	17	21	10	5	10	4	58	29
	10	11	18	7	8	1	1	5	36	15
	11		1		2				1	2
Anal soft rays	5				1					1
	6	4	1	6	4	2	3		11	9
	7	23	13	16	10	4	6		52	20
	8	6	14	10	6	2	2		30	10
	9		2						2	
Scales above lateral line, to second dorsal	2	1							1	
	3	–	2	6	9	2	7		8	18
	4	6	8	12	12	4	4		26	20
Scales below lateral line	6						4			4
	7		4	3	3		7	7	7	17
	8	7	5	9	14	6		9	21	29
	9	3	1	5	3				9	3
	10				1					1
Interorbital scales	0				2					2
	1	1			2		1		1	3
	2	4	5	1	1	2	2		10	5
	3	4	4	15	4	4	8		23	16
	4	1	1	3	9	2			5	11
	5				1					1

The coronal pore is described by Hubbs and Cannon (l.c.: 66) as occasionally covered with skin. In all of the populations examined the external opening of the pore was very much reduced or absent. Our data show it usually distinguishable in the populations from Jones, Salters, Singletary, and Waccamaw lakes

and usually absent in populations from White and Ellis lakes. The satisfactory determination of the presence or absence of the coronal pore is a delicate matter and requires very well preserved material. Most of the collections studied were made with the use of rotenone poison, and pore determination was definitely more difficult and sometimes impossible on this material. The statistical data on this one point should not be taken too literally.

In other meristic characters the variations seem to be mostly of a local nature. The supratemporal canal shows high percentages of incompleteness in the Salters and Singletary populations but not in the Jones or other populations. The average

TABLE 4

Comparison of the subspecies of Hololepis thermophilus *in several diagnostic characters*

		thermophilus oligoporus				*thermophilus thermophilus*				
		Jones	Salters	Singletary	Total	Total	White	Waccamaw	Ellis	Hubbs and Cannon (1935)
Pored scales	0–12	97.9	95.5	86.7	95.2	18.6	14.3	33.3	0	28.6
	13–21	2.1	4.5	13.3	4.8	81.4	85.7	66.7	100	71.4
Unpored scales	24–37	2.1	9.1	10.0	4.8	91.7	85.7	66.7	100	100
	38–59	97.9	90.9	90.0	95.2	8.3	14.3	33.3	0	0
Ratio of pored to unpored scales	0–.28	94.7	95.5	73.3	90.5	5.1	4.8	0	0	9.5
	.33–.88	5.3	4.5	26.7	9.5	94.9	95.2	100	100	90.5
Coronal pore	0	30.4	20.0	33.3	27.7	64.9	77.8	25.0	72.7	
	1	69.6	80.0	66.7	72.3	35.1	22.2	75.0	27.3	
Maximum size (mm.)		26.7	25.2	27.6	27.6	35.0	28.1	28.7	29.0	35.0

Population figures in percentage, except maximum size.

number of spines is fewest in lakes Salters and Waccamaw and highest in White Lake specimens, and the dorsal soft rays average fewest in Lake Waccamaw. Both dorsal and anal rays have a high average number in Salters Lake specimens. The number of scales above and below the lateral line are fewer on the average in Lake Ellis material. It will be noted that in most meristic characters the White Lake population is more variable than the others. This phenomenon is particularly apparent in the ratio of pored to unpored scales, infraorbital and operculomandibular pores, scales below the lateral line, and interorbital scales. This suggests that the ecological conditions in White Lake are such that a wider variety of genotypes are tolerated than in the very dark lakes.

It was suspected that the reduced number of lateral line pores in *oligoporus* might be a direct result of its smaller average size. Accordingly a careful tabulation of pore number and size was made for the Jones Lake population. Above

21 mm. in standard length no correlation was evident between number and length. Specimens smaller than 21 mm. from all lakes frequently revealed a near maximum number of pores, and the pores in juveniles, if visible at all, were equally evident on the anterior and posterior scales of the pored portion of the lateral-line row. It is concluded, therefore, that the full complement of lateral line pores is present at a standard length of 21 mm. for all populations, and probably at a considerably smaller size.

A slight sexual dimorphism is present in the number of pored scales in the lateral line, with the females tending to have a higher average number than the males. In Jones Lake, which is represented by the longest series of specimens, the females had 7.30 ± 0.55 pores, and the males 5.05 ± 0.46. The t-value of the difference is 3.02 for 94 degrees of freedom, which is highly significant. In Singletary Lake the difference between the means of 10.50 ± 1.01 for the females and 9.00 ± 0.72 for the males is not significant at the 5% level according to the t-value of 1.16 for 28 degrees of freedom. Very likely a larger series from Singletary would result in a significant sexual difference. Similar differences were not apparent in the other lakes on the basis of the limited material available. The sex of the specimens was recognized from the character of the genital papilla and the coloration of the vertical fins and belly.

In coloration and pattern there do not seem to be appreciable differences in the populations of *oligoporus*. The limited material from Lake Waccamaw does not appear to differ significantly from this pattern, although the specimens from the lake itself are generally pale. In the White Lake population (Plate 6, Figs. 1–8) the lateral blotches are more numerous, numbering 10–15 (average 13) instead of 8–13 (average 10); and the dorsal saddles are correspondingly more numerous, usually numbering 10 or 11 instead of 8. The pattern in the Lake Ellis material is difficult to evaluate. Some males (Plate 7, Figs. 2 and 8) have a very uniform coloration, whereas other specimens of both sexes may be very boldly marked on the body (Figs. 4, 9 and 10). In general the lateral blotches tend to be obscure or to coalesce, and a numerical expression of them becomes very arbitrary. Sexual differences in coloration and genital papilla are very poorly indicated in this July collection.

RELATIONSHIPS

The relationships of *t. oligoporus* are obviously with *t. thermophilus* of the same geographic region. In the principal characters distinguishing the two subspecies, *oligoporus* is the more specialized form, having fewer pored scales in the lateral line, fewer pores in the infraorbital series, and a lesser size (if the latter difference is genetic). Since these are all differences of degree only, it may be presumed that *oligoporus* has been derived directly from *thermophilus* rather recently, possibly since the time of the relatively dry oak-hickory maximum found in the sediments of Singletary Lake (Frey, 1951a).

There are certain biogeographical difficulties in the present interpretation of *Hololepis thermophilus*. The species is recorded only from North Carolina where it ranges widely over the upper and lower Coastal Plain. Typical *thermophilus*

is here recorded from lakes White, Waccamaw, and Ellis, each of which is in a separate major Atlantic drainage system. Subspecies *oligoporus* is recorded from lakes Jones, Salters, and Singletary, which are all within 8 miles of White Lake and are likewise located in the Cape Fear River drainage. There is, however, a dichotomy in this drainage, as explained by Frey (1951b), with Jones, Salters, and White lakes being closely associated in one arm, and Singletary and Black associated in the other. Yet, although colored lakes of both arms contain *oligoporus*, White Lake does not.

On first consideration one might argue that here is another example of the type of ecotypic variation described by Frey for a number of other species in these lakes. Careful analysis, however, shows a more complicated situation, in which the present conclusion is that *oligoporus* is a valid genetic subspecies.

In the first place, if the variations in numbers of pores were primarily ecotypic, then one ought to find the same variant in Ellis Lake, which is very similar to the *oligoporus* lakes in the dark color of its water, low pH, low concentrations of chemically active substances, and shallow depth. The population in Ellis Lake, however, is typical *thermophilus*.

In the second place, the pores concerned with the lateral line system, especially those of the various canals of the head, are not only relatively constant for a given species but also are an aid in interpreting phylogenetic relationships. The latter would not be possible if the characters were subject to marked environmental modification. It is true that the number of pored scales in the lateral line of these two subspecies parallels the similar dichotomous variation in *Perca flavescens* in these lakes (Frey, 1951b), but the total number of scales in the lateral line of *Hololepis* shows an exactly opposite trend. Moreover, each lake exhibits population characteristics that tend to be greater from one lake to another than are the ecotypic differences noted by Frey for certain of the other species in these lakes.

From the above discussion it may reasonably be doubted that *oligoporus*, the more specialized subspecies, is monophyletic in its derivation from *thermophilus*. The alternate theory of polyphyly would have *oligoporus* originating two or more times in convergent fashion in response to similar environmental selection. Under either theory the end result of two subspecies, each characterized by a high degree of genetic uniformity, would be the same. We find nothing inconsistent between the concepts of subspeciation and polyphyly, although recognizing that the latter origin of a subspecific unit is probably the exception rather than the rule.

It is well to recall the statement of Hubbs and Cannon that species of this genus should prove desirable aquarium fishes and hence are probably suited for an experimental investigation of any environmental control of the characters used in taxonomy. If future experimental work demonstrates that these characters of *Hololepis* are environmental variations of the species as a whole, then a complete restudy of *Hololepis* and a reevaluation of the sensory pore system as a taxonomic character will be necessary. Critical collecting in the lake outlets

and in the neighboring streams may do much to clarify the distribution and inter-relationships of the two subspecies.

Hololepis serrifer Hubbs and Cannon

Material examined

LAKE ELLIS, 5 specimens, 15 July 1950. Among weeds in shallow water.

These specimens agree well with the description given by Hubbs and Cannon (l.c.: 33–36). The five individuals vary from 31.5 mm. to 33.8 mm. in standard length. Dorsal fins IX, XI (4)–11 (2), 12 (2), 13; anal soft rays 5 (2), 6 (2), 7. Pored scales in lateral line 29, 30, 33, 35 (2); unpored scales in same row 16, 17, 19, 22 (2); total scales 46, 51, 52 (2), 57. Scales between lateral line and origin of second dorsal 4 (4), 5; scales below lateral line 9, 10 (3), 11. Infraorbital pores 6 (9), 7; operculomandibular pores regularly 9. Interorbital pores 0–0, 0–1, 1–1 (3).

Color pattern rather obscure. Lateral line marked by a conspicuous light streak. A fine dark streak along mid-line of sides along which are arranged a series of vague, dark, vertical bars. Median pair of caudal spots large and intense; dorsal and ventral ones scarcely apparent. Breast and belly immaculate. Vertical fins faintly barred on rays only. All appear of one sex, probably females.

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EXPLANATION OF PLATES

PLATE 6

FIGURES 1–4 males, 5–8 females, *Hololepis thermophilus thermophilus*, White Lake. Figures 1–6 collected Sept. 8, 1947; Figure 7, July 24, 1947; Figure 8, August 29, 1947.

FIGURES 9–13 males (9 holotype), 14–18 females, *Hololepis thermophilus oligoporus*, Jones Lake, collected Sept. 10, 1947.

PLATE 7

FIGURES 1–8 males, 9–10 females, *Hololepis thermophilus thermophilus*, Lake Ellis, collected July 15, 1950.

FIGURES 11–15 males, 16–19 females, *Hololepis thermophilus oligoporus*, Salters Lake, collected Sept. 9, 1947.

PLATE 8

FIGURES 1 male, 2–4 females, *Hololepis thermophilus thermophilus*, Lake Waccamaw. Figure 1 collected in main inlet, Aug. 9, 1947; Figures 2–3, Aug. 14, 1947; Figure 4, Aug. 18, 1947.

FIGURES 5–8 males, 9–11 females, *Hololepis thermophilus oligoporus*, Singletary Lake, collected Sept. 5, 1947.

PLATE 6

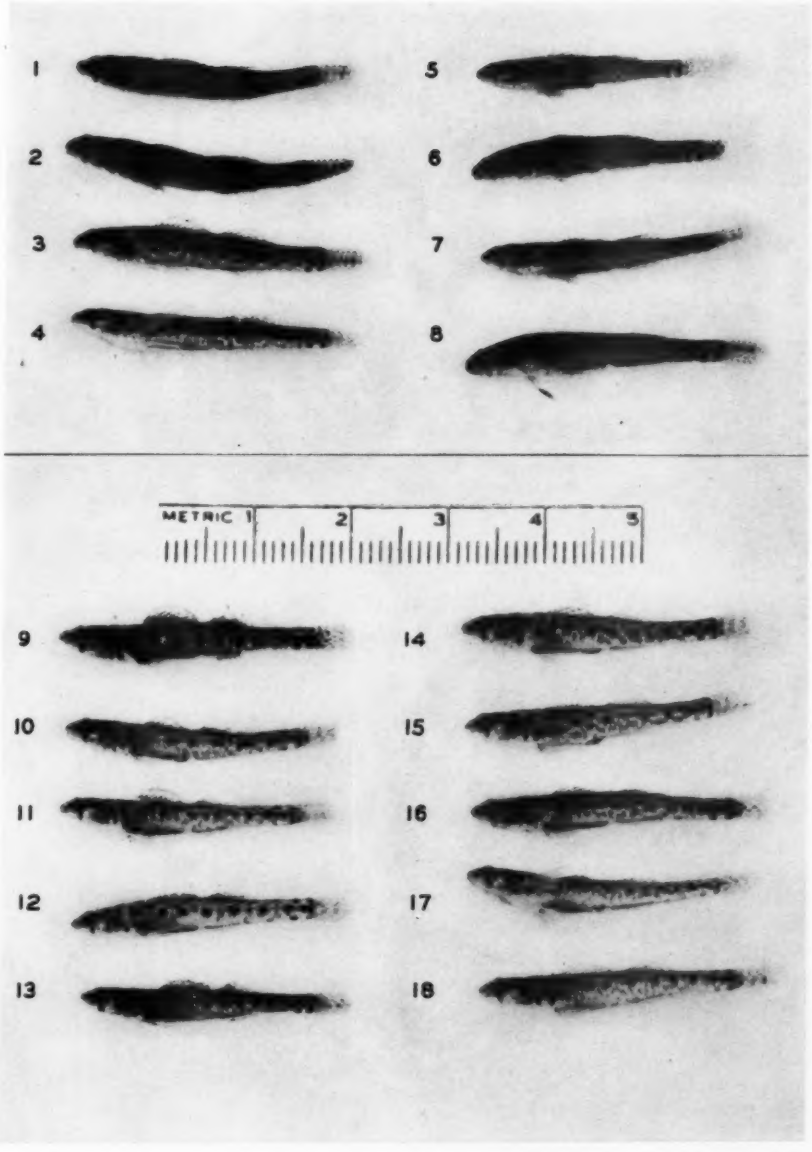


PLATE 7

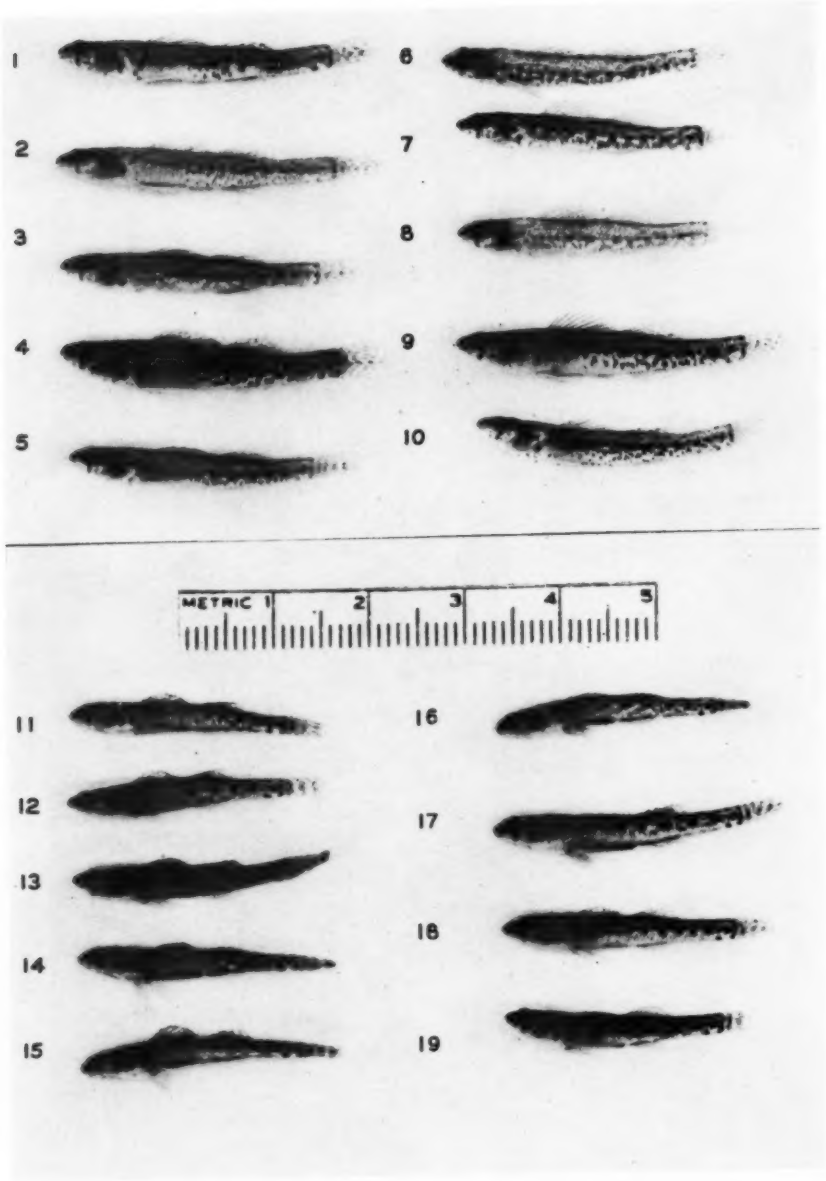
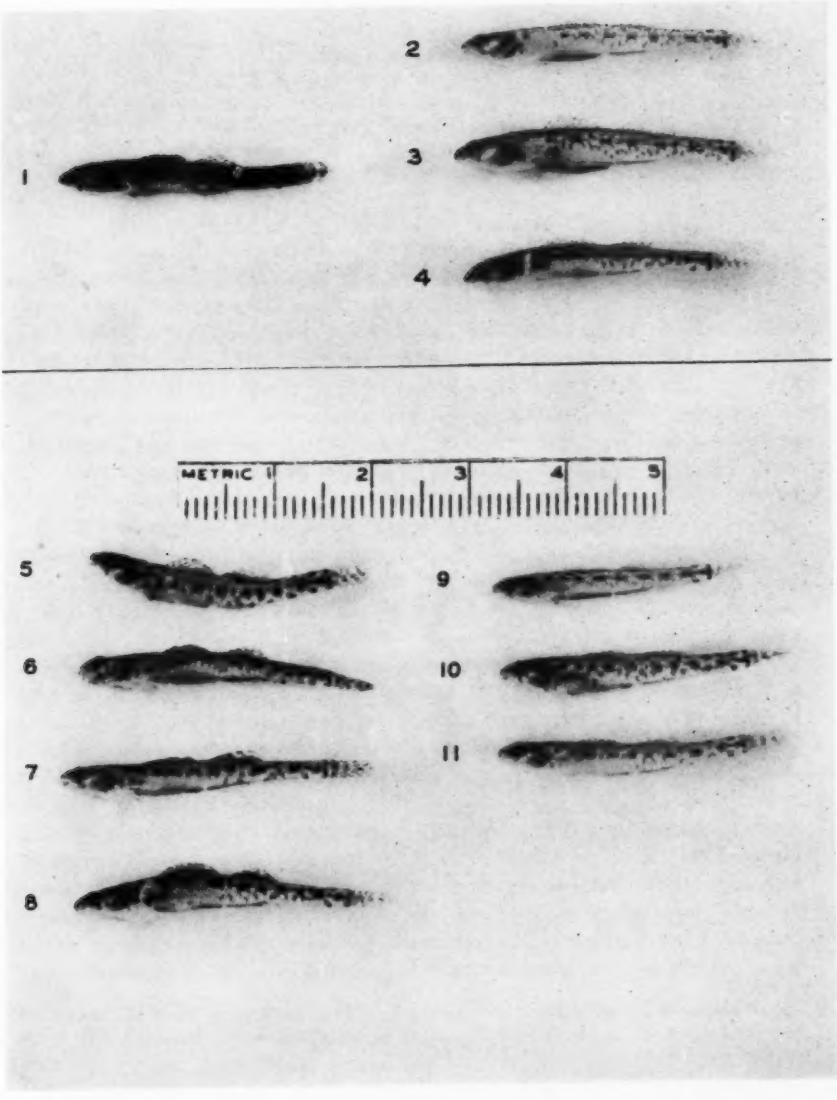


PLATE 8



ENCYSTMENT, EXCYSTMENT, AND THE NUCLEAR CYCLE IN THE CILIATE *DILEPTUS ANSER*[1]

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Plates 9–12

Introduction

It has been almost three centuries since the ciliate Dileptus was first observed. According to Dobell (1932), van Leeuwenhoek in 1676 sketched and described a form which was probably a species of Dileptus. Since this first description, comparatively few investigators have worked with the genus, probably because of cultural difficulties. The literature concerning this genus is full of contradictions, confusing generalities, and illustrations of dubious value.

The form used in the present study is *Dileptus anser*, which, according to Kahl (1935), was first described by O. F. Müller in 1786 as *Vibrio anser*. The genus Dileptus was established in 1841 by Dujardin. Kahl (1935, p. 205), gives the following synonyms of *D. anser: Amphileptus anser* and *A. margaritifer* Ehrb., *Dileptus gigas f. varsaviensis* Wrzes., and *D. irregularis* Maskell.

It seems quite apparent from their descriptions and illustrations that Prowazek (1904), Visscher (1923, 1927a, 1927b), and Studitsky (1930) were using *D. gigas* as a synonym of *D. anser*. Hayes (1938) has made mention of this. From Kahl's description of *D. gigas* and *D. anser*, there are several differences between the two species. *D. gigas* has a sausage-shaped macronucleus, a body length of 1–1.6 mm., and a relatively short proboscis which terminates in a papilla. In contrast, *D. anser* has a multipartite macronucleus, a body length of 250–600μ, and a proboscis which varies from one-half to one times the body length. The species of Dileptus used in this study agrees with Kahl's description of *D. anser* (Müller) Dujardin.

Though descriptions of the encystment process and the nuclear cycle of *D. anser* have appeared in the literature previously, their lack of completeness and the presence of errors call for a further examination of these phenomena. The excystment process of this form has never been previously described. Thus, it has been the purpose of this research (1) to repeat and evaluate some of the work of previous investigators, (2) to establish a workable culture method for those

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who follow, (3) to describe the encystment and excystment processes and (4) to contribute some new data concerning the nuclear cycle with special reference to encystment and excystment.

CULTURE METHODS

Pond water from which the ciliates were originally isolated was collected and food organisms were removed by means of a Foerst continuous centrifuge. The centrifuged pond water was stored in stoppered 1-liter Pyrex flasks at 15°C. until needed. Water treated in this manner was used after twelve months of storage without ill effects.

Previous investigators, among them Allescher (1912), Visscher (1923) and Hayes (1938), have commented on the wide variety of food which *D. anser* will eat. Undoubtedly these observations are accurate, but there seems to be a preference for Euglena and Halteria. The use of Euglena is the less desirable of the two since such great numbers are ingested by Dileptus that they render nuclear observations unusually difficult in the stained animal. Halteria, on the other hand, has a large macronucleus which stains heavily and is easily differentiated from the macronuclei of Dileptus. The single micronucleus of Halteria also differs from the micronuclei of Dileptus. These differences will be discussed in detail. *Halteria grandinella* (Müller) cultures were started by isolating about fifty individuals from pond water collected where Dileptus was found. These halteriae were washed in centrifuged pond water and cultured in a 0.5 percent timothy hay infusion made up in tap water. Wild bacteria soon contaminated the hay infusion, thus supplying suitable food for the halteriae. Subcultures were made at 5-day intervals to 600-cc. volumes of fresh infusion. These cultures were grown at room temperature and flourished from 10 to 30 days.

Dileptus cultures were begun by isolating several individuals in pond water. Each culture was fed washed halteriae daily. This was accomplished by centrifuging 50 cc. samples of flourishing Halteria culture. The concentrated halteriae were drawn off rapidly, placed in 50 cc. of pond water and centrifuged a second time. Each drop of concentrate contained hundreds of halteriae which distributed themselves gradually when introduced dropwise in the individual cultures of Dileptus. The amount of food given daily was from 2 to 5 drops of concentrate per Dileptus culture, and with few exceptions, all the halteriae were consumed by the next feeding. It is indeed a striking sight to see *D. anser* feed. The halteriae are attacked and devoured with greedy abandon. It is not unusual to see three or four halteriae stuck to the proboscis at one time, passing toward the cytostome by ciliary action of the proboscis. The food organisms are literally packed in the endoplasm until the dileptus resembles a bag full of marbles.

Using the above method, mass cultures were kept in finger-bowls from which pure-line cultures were started when desired. Such mass cultures have been maintained for three years. Subcultures were started about once a week, even though a finger-bowl culture will flourish a month or longer if fed regularly. Dileptus is not an easy animal to culture and not all subcultures were successful. Reasons for this are somewhat elusive. At any rate, since a successful culture was not positively assured, it was expedient to keep at least five mass cultures

going at all times and to isolate several pure-line cultures when only one or two were actually required. The difficulties of culturing this animal are common knowledge to those investigators who have attempted it; for example, Visscher (1925b, p. 406) says: "Of more than a hundred cultures which were started, all but one died off within a few months."

MOUNTING TECHNIQUES

Numerous fixatives were tried during this investigation to determine (a) the best fixative to use on Dileptus, and (b) the possibility of one fixative preserving a specific organelle better than another. Schaudinn's with acetic acid, Carnoy's, Kahle's, and Champy's fluids proved superior from the standpoint of over-all fixation, i.e., the preservation of nuclear detail with a minimum of body distortion. Fixatives which did not kill rapidly could not be used, since *D. anser* is extremely sensitive to toxic mixtures and reacts violently to such substances. Such a reaction is manifested by rapid, backward swimming, rupturing or casting off of the proboscis, and finally by total disintegration.

Upon fixation, the cuticle of *Dileptus anser* becomes sticky and adheres readily to a clean coverslip. Large groups of animals become entangled upon fixation, thus making studies of individual animals impossible. For this reason, only five animals were used in a coverslip preparation. Each animal was isolated on the coverslip in a droplet of water and killed individually with a drop of fixative.

When sections were to be made, two techniques for imbedding were used. (1) The technique described by Beers (1937) was used when animals were to be sectioned *en masse*. (2) Individual animals were oriented and imbedded by the following technique. Sheets of membrane were stripped from the inner surface of a hen's egg. These were fixed in formalin, washed, and stored in 70 percent alcohol colored with Fast Green until needed. Wedge-shaped strips, 1 mm. wide at the apex, were cut and served to manipulate the animals. Several dilepti were fixed in a Boveri dish of Schaudinn's fluid. An individual, when gently detached from the dish, became adherent to the egg-membrane wedge. The specimen's orientation on the wedge was noted. With the egg membrane serving as a lifter, the animal was dehydrated and imbedded. The wedge was clearly visible after imbedding; hence the dileptus could be sectioned serially in any desired plane. Sections of the egg membrane usually appeared in the finished preparation, but these were not objectionable.

The stains used with best results were Feulgen with Fast Green, Carmalum with Lyon's Blue, Mallory's triple stain, and Heidenhain's iron haematoxylin. The nuclei of *D. anser* leave much to be desired in their ability to take and retain a stain. The multinucleate condition may account for the apparent chromatin deficiency, since the nuclei of other multinucleate forms such as Opalina and Pelomyxa exhibit a similar lack of stainability due to a low chromatin content.

THE PROCESS OF ENCYSTMENT

Deficiencies and errors in the literature concerning the encystment process of *Dileptus anser* necessitate a clarification of this phenomenon. For example, Cienkowsky drew an illustration of a *D. anser* cyst which appears in Bronn

(1887–1889, Tafel LIX, Fig. 4g.). This figure shows a cyst with two membranes the endocyst being fastened within the ectocyst by means of bipolar attachments. Actually, my observations show conclusively that the cyst of *D. anser* has no bipolar attachments; thus Cienkowsky did not draw the cyst of this species. Cienkowsky, according to Prowazek (1904), observed both encystment and excystment, but I have been unable to find an account of either in the literature. Such observations would be of dubious value if they were made on the cyst which Cienkowsky illustrated.

Prowazek (1904) gives an account of the encystment process in a species of Dileptus which he does not identify. From Figs. 1 and 2, p. 65, the form described appears to be *D. anser*. In this account, the author describes the ectocyst as being yellowish in color and reticulated. It will be shown later that neither of these observations is correct. Prowazek also mentions a plug ("Propf") by which the cyst is attached to the substrate. I have never seen this structure, and its existence is highly unlikely. Adhesiveness is inherent in the ectocyst material, and no special structure or plug is needed to attach the cyst to the substrate. During cyst-membrane formation, Prowazek describes the formation of reduction bodies which were proliferated from the cytoplasm following ectocyst formation. I am unable to confirm this observation.

Hayes (1938) followed encystment no further than the formation of the ectocyst. His main interest concerned the nuclei within the cyst. This phase of the problem will be discussed later along the with nuclear cycle.

In the present study, encystment was brought about with a fair degree of regularity by placing ten animals on a supported coverslip and feeding regularly as needed. The coverslip was supported by a 6-mm. square cut from a standard microscope slide. Microscope slides bearing two supported coverslips were kept in moist chambers and examined daily for cysts. Using this method the age of the cysts could be determined with an accuracy of plus or minus 12 hours. The animals invariably divided many times so that when encystment occurred in from one to five days, many cysts were present. The coverslips formed an excellent substrate for cyst attachment and provided an efficient tool for handling groups of them.

Though studies on the causes of encystment in *D. anser* are not yet completed, there are indications that encystment results from the gradual accumulation of this ciliate's own excretion products. The supported coverslip method for inducing encystment allows a small number of animals to reproduce and, over a period of several days, to become crowded. This crowding, in turn, is responsible for the production of gradually increasing amounts of excretion products. When initial crowding was brought about in an attempt to induce encystment, all the animals in the culture died. I attribute this to a too rapid accumulation of excretion products. On the other hand, crowding does not induce encystment when the medium is changed constantly over a period of five days. In this case, the constantly changing medium prevents the accumulation of excretion products. Thus, it may be concluded that crowding *per se* is not responsible for encystment in this species, but rather the accumulation of excretion products which result from the crowding.

D. anser forms only resting or protective cysts. In the living condition, the cysts are spheroidal; they have a light tan color which becomes darker as the cyst ages. There are three cyst membranes, ecto- meso-, and endocyst; the color is present only in the mesocyst. Hayes (1938) found the diameter of dilepti cysts to be 80μ. This is a good average, though cysts vary somewhat in over-all size and shape. Cysts measuring as little as 65μ have produced normal, viable animals upon excystment.

The first indication of encystment which may be observed in the living animal is a change in the consistency and color of the cytoplasm. In the active specimen, the cytoplasm appears granular, and if the animal were recently fed, vacuolated. Prior to encystment the cytoplasm becomes homogeneous, tan in color, and hyaline in appearance. Food vacuoles disappear and only the dorsal row of contractile vacuoles interrupts the homogeneity. Visscher (1923) mentions the two physical states of the protoplasm, but assigns no significance to either. Prowazek (1904, p. 64) says, "Vor der Encystierung wird das Plasma des Dileptus durch Wasseraufnahme trübe und das Tier büszt etwas von seiner schlanken, zierlichen Gestalt ein,—der Körper wird eiförmig und an einer Seite ragt der bewegliche Rüssel hervor." On the contrary, water must be lost during the encystment process, not taken up as Prowazek postulates. Actually the passage of water in or out of the animal cannot be observed, but the encysted animal has a much smaller volume than the precystic individual. Since there is no loss of protoplasm during the encystment process, it is reasonable to conclude that water is lost, causing the protoplasm to become more dense and thus turbid as Prowazek mentioned.

A second overt precystic sign is the change in body shape (Figs. 1 and 2) which occurs simultaneously with the cytoplasmic change. The proboscis becomes blunt at the tip and is gradually resorbed into the body causing the latter to assume a plumpness which is atypical of the trophic animal. These changes in body shape occur gradually, but over a relatively short period of time. Figure 1 shows an early precystic form. Its circumference is greater and its length shorter in comparison to these dimensions in the trophic animal. This is quite obvious when the specimen of Fig. 1 is compared to the trophic animal in Fig. 6. Figure 2 shows the same animal drawn 48 minutes later. There is a noticeable thickening and shortening of body and proboscis. The pharyngeal basket and cytostome, though not shown, are still present at this stage. The trichocysts are still functional. This is evidenced by the fact that Halteria reacts strongly upon coming in contact with the ventral surface of the proboscis It is only along this surface that trichocysts are to be found. Halteriae react to the trichocysts of *D. anser* either by spinning violently and eccentrically away or by becoming partially cytolyzed and dropping stunned to the bottom of the dish. Though the trichocysts are functional, Dileptus at this stage makes no effort to feed, even in the presence of abundant food organisms. Thirty minutes later Fig. 3 was drawn. With the exception of a small proboscis remnant, the animal has assumed a spherical form. At this stage the contractile vacuoles cease to lie in dorsal alignment, and movements of the animal are restricted to a slow rotation in one locus. This rotation continues until the proboscis is completely resorbed and the animal becomes a

perfect sphere, or nearly so. Frequently the cysts are slightly elongate or irregularly shaped. Twenty minutes after Fig. 3 was drawn there were evidences of ectocyst formation. The ectocyst, in the first stages of its formation, is completely transparent, but its presence is detectable by small irregularities which produce indentations in the encysting animal as it rotates past them. The animal continues to rotate slowly, laying down extremely thin layers of ectocyst until the desired thickness is attained. The thickness of the ectocyst is not constant, but varies from 2 to 7μ. One cannot orient the thick and thin areas of the ectocyst with respect to specific structures, as all visible landmarks have disappeared by this time. Briefly then, the ectocyst is a colorless, laminated membrane and not yellow in color or reticulated as claimed by Prowazek (1904).

A close inspection of ectocyst formation has led me to conclude that this process is a modification of a similar process in the trophic animal. A trophic specimen of *D. anser* is frequently attached to some object or to the substrate by a caudal thread. This thread prevents forward motion, but permits the animal to rotate on its longitudinal axis. The substance which forms this thread appears to be exuded by the entire body of the animal and passed by ciliary action to the posterior end where it is spun into a thread by the animal's rotation. During locomotion the thread may be seen trailing behind for a distance up to twice the body length. Bits of debris coming in contact with the animal's body, as well as stunned food organisms, may be seen to pass along the body and come to lie on the caudal thread which is obviously sticky (Figs. 6 and 7). Such a substance secreted by the body may account for the stickiness of the fixed animal. The caudal thread also appears to serve as a sea anchor (Fig. 6) and to retard the forward motion of the animal. This retardation of forward motion allows the animal to rotate many times in each millimeter of distance traversed and consequently to search the area more thoroughly in its quest for food. The secretion of a caudal thread ceases at the onset of the precystic phase. Is there a plausible explanation for the cessation of this singular activity? I believe there is, and it may be arrived at by a comparison of the caudal thread and the ectocyst. It will be seen from Figs. 6, 7, 11, and 12 that both structures are almost completely transparent. It is quite obvious that both are exceptionally sticky; otherwise food organisms and debris would not adhere to the caudal thread, and the cyst would not adhere tenaciously to the substrate. Both substances are exuded from the surface of the cell. Figure 7 shows the formation of the caudal thread; the ectocyst is produced in the same manner, although the direction in which it is laid down is at variance. Faced with these facts, there can be little doubt that the caudal thread and the ectocyst are composed of the same material, secreted in the same manner. Taking this assumption to be true, it is only reasonable to conclude that *D. anser* conserves the ectocyst material during the precystic phase. In the absence of feeding, no new ectocyst material can be synthesized; thus some must be held in reserve to be used when rounding up is complete.

The mesocyst is formed next. This pigmented middle membrane is almost 2μ in thickness. At one point there is a slight depression which Prowazek (1904) claimed was derived from the old cytostome. Hayes (1938, p. 20) says: "In the

mature cyst there is one point at which the wall is quite thin and it is at this point that the animal emerges." This statement is somewhat misleading in that the animal does not emerge through the thin area, but rather the cyst begins to crack open at this point. The animal actually emerges through a large fissure as shown in Fig. 21.

It is impossible to state when the endocyst is formed since it cannot be seen in the resting cyst due to the color and thickness of the mesocyst. This third cyst membrane can only be seen during the process of excystment. For this reason, the presence of the third cyst membrane has never been previously reported in this species.

THE EXCYSTMENT PROCESS

The search which I have made of the literature concerning *Dileptus anser* has failed to uncover a description of the excystment process. In all probability, neither induced excystment nor excystment from natural causes has ever been previously described in this species. It is felt that such a description will add to our limited knowledge of this form.

In the course of this study, it was found that a 0.125 percent wheat infusion would induce 100 percent excystment in young cysts, i.e., cysts not over three months old. This infusion was used whenever photographs and drawings were to be made of the excystment process. The infusion was made by heating 100 cc. of distilled water to a temperature of 90°C. To this, 0.5 gm. of crushed wheat was added and infused for five minutes at this temperature. After filtering through cotton, 10 cc. of the filtrate was diluted with 30 cc. of distilled water.

Coverslips upon which the ciliates had encysted were inverted over the depressions of Columbia culture dishes and enough excystment medium was pipetted in at the side to cover the cyst-surface of the coverslip. These preparations were kept in a moist chamber until observations were to be made. This method allowed a sufficient volume of excystment medium to be used, and at the same time, provided a flat, dry surface through which the cyst could be observed and photographed under the compound microscope.

Cysts usually remained in the excysting medium for 4.5 hours before the first signs of activation were observed. Contractile vacuole activity and a very slow cyclosis constituted the first signs of excystment. Figures 12 and 17 show a cyst in the early stages of excystment. The thick, colorless ectocyst can be seen closely investing the pigmented mesocyst. This, in turn, appears to be in contact with the animal, although an endocyst is present. However the endocyst cannot be seen in the resting cyst or in the early stages of excystment. A large contractile vacuole can be seen in both figures. Contractile vacuole activity continues; the animal swells, rupturing the outer cyst membranes, and the swollen animal tightly invested by the thin endocyst can be seen through the fissure. Figures 14 and 18 show the ruptured cyst, with the animal protruding slightly through the opening toward the observer. Two large contractile vacuoles are visible. Elevation of the endocyst occurs at this time. Figures 13 and 19 show slightly elevated endocysts. The mesocyst is shown in optical section in these figures, and

appears more heavily pigmented than is actually the case. Contractile vacuole activity increases gradually and differentiation begins at this stage. Cilia appear and the animal begins to spin slowly within the endocyst. The endocyst now increases in diameter, and the animal increases its speed of rotation (Figs. 15 and 20). The animal in Fig. 15 appears as a blur due to the rapidity of its rotation. This photograph clearly demonstrates the three cyst membranes. Figure 20 shows in side view an animal which has elongated sufficiently to double on itself. This figure, which shows the endocyst well, was drawn 5 hours and 20 minutes after the cyst had been placed in the excystment medium. Five minutes later, the animal had the appearance shown in Figs. 16 and 21. The proboscis has begun to differentiate and the endocyst has become greatly enlarged, spreading the 2 cyst halves widely apart. Here again the three cyst membranes are clearly shown. At this stage of excystment, the animal pushes against the endocyst, first with its proboscis, then with the pointed caudal end, in an effort to be free of the endocyst. Finally the endocyst ruptures and the animal escapes. It requires from 5.5 to 6 hours for the completion of excystment.

The newly excysted animal is easily differentiated from the mature trophic animal. There are three conspicuous features by which the newly excysted *D. anser* may be recognized. The shape of the proboscis is perhaps the most obvious in that it is shortened and bears a blunted tip (Fig. 22). The cytoplasm of the entire organism is tan and hyaline-like in appearance. These two characters strongly reflect the appearance of the early precystic animal as shown in Fig. 1. A third, less obvious difference is the arrangement of the contractile vacuoles. At this early stage they are arranged more or less irregularly along the body. Later, they become aligned along the dorsal margin, extending from the base of the proboscis to the posterior end. Figure 22 shows an animal 5 minutes after its emergence from the cyst. This figure was drawn from a prepared slide since the constant movement of a living Dileptus prohibits the use of a camera lucida. The general shape of the body was preserved, but the proboscis was twisted 90° out of line with the body. For this reason, the cytostome can be seen in the mid-line with the ciliated feeding groove leading into it from an angle. When viewing the animal ventrally, one sees a straight feeding groove running in the mid-line of the proboscis.

Hyman (1940, p. 52, Fig. 8) shows an animal which she calls *Dileptus gigas*, though on p. 172 she refers to it as *Dileptus anser*. The contractile vacuoles in this figure are aligned along the ventral surface of the animal and bear a strong resemblance to those of a newly excysted specimen. The contractile vacuoles of *D. anser* are always situated dorsally in the adult trophic animal. Hyman's figure shows a distributed macronucleus and is thus *D. anser*.

THE NUCLEAR CYCLE OF DILEPTUS ANSER

D. anser feeds almost entirely on small protozoans. In stained specimens of healthy, well-fed dilepti, the nuclei of ingested food organisms stain readily, and when interspersed among the numerous nuclei of *D. anser* present a confusing picture. For this reason it was advisable to use only one species of food organism and to become thoroughly familiar with the nuclei of the species selected. The

nuclear apparatus of the food organism, *Halteria grandinella*, is easily differentiated from that of *D. anser*. Figure 4 shows the single micronucleus (MI) of *H. grandinella*; it measures 2.7μ in diameter and stains heavily with any of the nuclear stains tried. The position of the micronucleus in the cytosome is quite variable, lying either free in the cytoplasm or intimately enfolded in the macronucleus. The macronucleus (Fig. 4, MA) also stains deeply and contains chromatin granules of varying size scattered in the nucleoplasm. This nucleus may best be described as being massive; its shape is quite irregular, and may appear elongated, spheroidal, folded or twisted. Hence, measurements of the macronucleus are of little value. For comparison, a micronucleus (Fig. 5, MI) and two macronuclei (MA) of *D. anser* are shown. Invariably when *H. grandinella* and *D. anser* are fixed and stained together, the nuclei of Halteria stain more intensely. Hence, the depth of staining, as well as the great difference in size, serves to differentiate the nuclei of one species from those of the other.

The micronuclei (Figs. 5 and 23, MI) of interphase, trophic specimens of *D. anser* are spherical and agranular; they vary from 1 to 1.8μ in diameter. Surrounding each spherical, Feulgen-positive micronucleus is a halo of achromatic protoplasm (Fig. 23, MH) which is approximately 3μ in diameter. The number of micronuclei per individual is probably variable. The counting of these minute bodies is made difficult, and probably inaccurate, by the great number of macronuclei which are scattered in the cytoplasm. Hayes (1938) says, "The exact number of these bodies (micronuclei) was not determined but there are approximately twenty in each cell." My observations confirm this since I have never found less than 16 micronuclei in any individual examined.

The macronuclei (Figs. 5 and 23) of the interphase, trophic animal are variable both in size and number. In general, they are disc-shaped, but may be oval or somewhat irregular in outline. Each vegetative macronucleus is approximately 4.5μ in diameter, and there are always more than one hundred present in each individual. Visscher (1927b) states that the macronuclei number from several hundred to several thousand and that they vary from 0.1 to 1.0μ in diameter; Studitsky (1930) claims that the macronuclei vary from 180 to 400 per individual; Hayes (1938) states that there are up to 200 macronuclei per individual. These incongruous opinions attest to the variability of macronuclear number.

In the interphase macronucleus, from 1–4 Feulgen-negative bodies are present, each lying in a clear space surrounded by Feulgen-positive nucleoplasm (Fig. 24). Similar bodies in the macronuclei of other forms are variously called in the literature Binnenkörper, endobasal bodies, and karyosomes. I have chosen the word karyosome to identify the intranuclear body since it implies location without reference to function or composition. Calkins (1933, p. 51) says: "The distributed granules of deeply staining material which represent the substitute for a nucleus in *Dileptus gigas* are similarly composed of a plastin core and a chromatin cortex,...." Since *D. gigas* does not have a distributed nucleus, Calkins is referring to *D. anser*. His Fig. 25, p. 52 (*loc. cit.*), clearly shows the distributed nucleus. The apparent absence of a nuclear membrane led Calkins to use the term "granules" rather than macronuclei.

Fixed nucleoplasm is composed chiefly of extremely fine Feulgen-positive

granules. Hayes (1938, p. 15) says: "The chromatin (of the macronucleus) occurs in very fine granules, so fine as to appear almost as a homogeneous substance." It is interesting to note that the granular nature of the fixed nucleoplasm never changes regardless of the nuclear state, i.e., whether interkinetic, divisional, or encysted.

The nuclei of Dileptus cannot be observed in the living condition due to the similar indices of refraction of the nucleus and cytoplasm. For this reason, the study of the nuclear changes accompanying division is based on observations involving several thousand animals fixed and stained by one of the methods mentioned above. The sequence of events can be reconstructed with reasonable accuracy, however, by fixing in early, intermediate and late stages of cytosomal division (Figures 8, 9, and 10). These stages are easily recognizable under a dissecting microscope.

Fission is initiated by a rapid micronuclear division (Figs. 24, MI). The micronuclei enlarge, then elongate and finally divide, resulting in two full-size daughters. At no time were chromosomes or mitotic figures observed in them. However, Visscher (1927b) counted twenty-four chromatin granules in a pronucleus of a conjugating Dileptus. He considered them to be chromosomes and states that there are probably twenty-four pairs of chromosomes in the trophic nucleus.

At the elongation stage of micronuclear division, the macronuclei (Fig. 24, MA) begin to enlarge and the karyosome disappears. Studitsky (1930, Figs. 19, 20, 21, and 22) indicates the karyosome by a complicated reticulum bearing plastin granules. He claims that the nucleolus (karyosome) divides until there are from 20–40 nucleoli in the macronucleus, which in turn divides. My observations fail to confirm either the reticulate karyosome or a karyosomal division prior to macronuclear division. Figure 25 shows two macronuclei which have lost their karyosomes and have just begun to elongate. Figure 27 shows two macronuclei elongating. This is a relatively early stage, as elongating nuclei frequently attain a length of 100μ. When the nucleus reaches its maximum length, division occurs. The site of division of the nuclei bears no relation to the plane of fission of the cytoplasm; the dividing nuclei, quite like the interphase nuclei, are scattered throughout the cytoplasm.

Hayes (1938) claims that there is always some macronuclear division going on in the trophic animal. My observations do not confirm this, but may in part explain the observation of Hayes. Many animals have been observed in which only several macronuclei were beginning to elongate, whereas the remainder of the nuclei were disc-shaped. A closer scrutiny of these non-dividing nuclei showed them to have lost the karyosome in anticipation of division. It is not unusual to find in an individual that the majority of macronuclei lack karyosomes though some few still retain them. The few nuclei which retain karyosomes in the face of an approaching division probably exhibit a "nuclear lag" (Fig. 26, MAL). Since karyosomes are lost just prior to division and reformed following division, one must judge the direction of nuclear transition by the gross appearance of the animal if it contains nuclei both with and without karyosomes. A recently divided individual is invariably shorter than a typical trophic animal and it has an in-

completely formed caudal process or proboscis, depending on whether it is the anterior or posterior daughter.

On numerous occasions bizarre divisions were observed in which the nucleus was trefoil in shape. Nuclei dividing in this fashion begin division as triangles (Fig. 25) and elongate at the angles, resulting in Y-shaped figures. Though there are usually several such figures in each dividing animal, one may only speculate as to the normality of such a phenomenon. A second condition frequently encountered during elongation is torsion. Figure 26 shows two such nuclei which have just begun to elongate.

Nuclear studies on precystic animals reveal distinct changes in both the micro- and macronuclei. The micronuclei begin to decrease in diameter (Fig. 28) and reach their minimum size in the cyst (Fig. 29). No micronuclear reorganization was observed in the cyst. The macronuclei of the precystic animal show a marked decrease in size and become irregularly shaped. The karyosome becomes proportionately reduced in size. The micro- and macronuclei of excysting animals are also reduced in size, but before escape is made from the endocyst both micro- and macronuclei assume trophic proportions. Figure 30 shows one micronucleus and two macronuclei drawn from an excysting animal.

It seems reasonable that nuclear shrinkage should occur during encystment, for if water is lost from the cytoplasm, it must also be given up by the nuclei to preserve osmotic equilibrium. During excystment, when water is taken up by the cytoplasm, the nuclei again return to full trophic size.

SUMMARY

Dileptus anser (Müller) Dujardin was cultured in defaunated pond water to which washed specimens of *Halteria grandinella* were added daily as food organisms.

Encystment was induced by placing ten animals on a supported coverslip in a moist chamber and allowing them to feed and divide, thus becoming crowded gradually.

The cyst of *D. anser* has three membranes, an outer colorless, laminated ectocyst, a middle pigmented mesocyst, and a thin inner endocyst.

Excystment was induced by placing cysts in 0.125 per cent wheat infusion made up in Pyrex-distilled water. From 5.5–6 hours are required at 25°C. for the completion of excystment.

Each specimen of *D. anser* contains a minimum of 16 micronuclei and a minimum of 100 macronuclei. The micronuclei are Feulgen-positive spheres 1–1.8μ in diameter and are surrounded by an achromatic halo 3μ in diameter. The macronuclei of the interphase trophic animal are disc-shaped and average 4.5μ in diameter. Each macronucleus contains from 1–4 Feulgen-negative karyosomes which disappear prior to nuclear division. During cytokinesis micronuclear division is rapid and precedes macronuclear division.

In the precystic and encysted individuals the micro- and macronuclei are diminished in size probably due to water loss. During the excystment process the micronuclei and macronuclei return to full trophic size.

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EXPLANATION OF PLATES

PLATE 9

FIGS. 1–3. Precystic forms of *Dileptus anser* drawn from life; camera lucida. × 207.

FIG. 1. Early precystic animal.

FIG. 2. Same animal 48 minutes later.

FIG. 3. Same animal drawn 30 minutes after Fig. 2.

FIG. 4. Nuclear apparatus of *Halteria grandinella* (Schaudinn-Feulgen preparation; camera lucida). × 3388.

FIG. 5. A micronucleus and two macronuclei of *D. anser* (Schaudinn-Iron haematoxylin preparation; camera lucida). × 3388.

FIGS. 6–7. Drawings of the caudal thread of *D. anser* (Schaudinn-Iron haematoxylin preparations).

FIG. 6. A trophic *D. anser* using the debris on the caudal thread as a sea anchor. × 282.

FIG. 7. Posterior end of *D. anser* showing the width of the caudal thread before spiraling. × 376.

FIGS. 8–10. Drawings of division in *D. anser* (Schaudinn-Feulgen-Fast green preparations). × 282.

FIG. 8. Early division stage showing small proboscis bud in lateral aspect.

FIG. 9. Early division stage showing typical hourglass shape when viewed from ventral aspect.

FIG. 10. Late division stage showing enlarged proboscis bud in lateral aspect.

CT, caudal thread; CV, contractile vacuoles; FV, food vacuole; H, Halteria; MA, macronuclei; MI, micronuclei; PB, proboscis bud; TR, trichocysts.

PLATE 10

FIGS. 11–16. A series of photographs showing the excystment process of *Dileptus anser*. Figs. 12–16 from life. × 530.

FIG. 11. Resting cyst (Schaudinn-Feulgen preparation). Ectocyst shows faintly due to clearing action of damar. Mesocyst, granular nature of the cytoplasm, and some macronuclei are prominent.

FIG. 12. Early excystment stage showing a single large contractile vacuole. Ectocyst and mesocyst show well. Eventual point of cyst rupture is indicated by thin area in the mesocyst.

FIG. 13. Lateral view of open cyst. The three cyst membranes show clearly. The endocyst closely invests the animal as it protrudes through fissure. Several contractile vacuoles are evident.

FIG. 14. Front view of stage shown in Fig. 13. Numerous contractile vacuoles are present.

FIG. 15. Late stage of excystment showing animal spinning within the endocyst. Cyst halves have been forced far apart by enlargement of endocyst.

FIG. 16. Final stage of excystment showing the dileptus trying to rupture the endocyst which at this stage protrudes through the open cyst halves.

EC, ectocyst; EN, endocyst; MC, mesocyst.

PLATE 11

FIGS. 17–22. Successive stages in the excystment process of *D. anser*.

FIGS. 17–21 redrawn from photographs. FIG. 22 drawn from prepared slide.

FIG. 17. Early excystment stage, the onset of activation.

FIG. 18. Front view of opened cyst.

FIG. 19. Lateral view of opened cyst.

FIG. 20. Lateral view of differentiating animal within endocyst.

FIG. 21. Late excystment stage showing greatly enlarged endocyst and well differentiated animal.

FIG. 22. Differentiated animal five minutes after emergence from cyst. Contractile vacuoles have not yet aligned in dorsal row. (Schaudinn-Carmalum-Lyon's Blue; camera lucida).

CV, contractile vacuole; CY, cytostome; EC, ectocyst; EN, endocyst; MC, mesocyst.

PLATE 12

FIGS. 23–30. Micro- and macronuclei selected from individuals of *D. anser* in various stages of division and cystment (Schaudinn-Iron haematoxylin; camera lucida).

FIG. 23. Nuclei of trophic individual.

FIG. 24. Early division stage showing micronuclear enlargement and division.

FIG. 25. Early division stage. Triangular shaped nucleus will become Y-shaped; karyosomes absent.

FIG. 26. Early division stage showing torsion and macronuclear lag.

FIG. 27. Intermediate division stage showing macronuclear elongation.

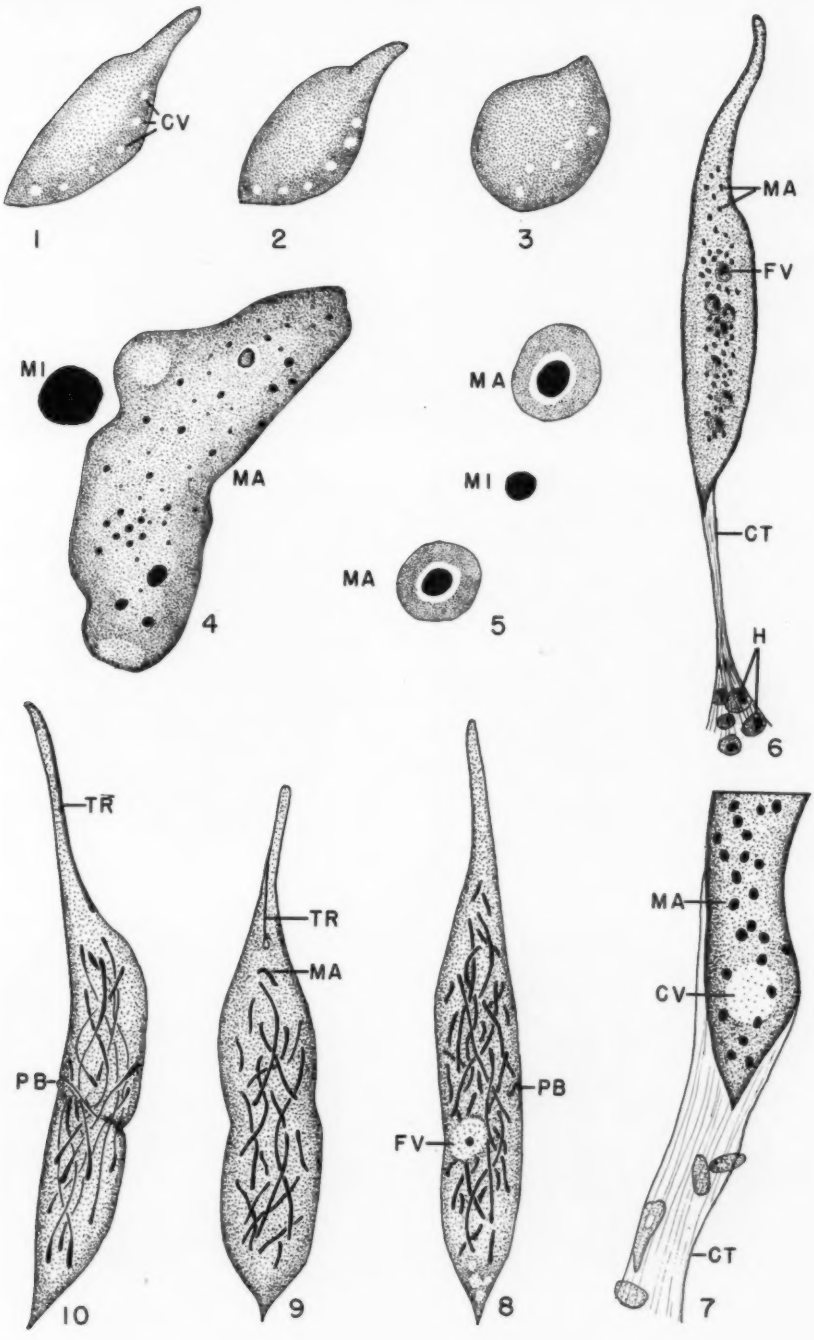
FIG. 28. Typical nuclei from a precystic animal showing decrease in size of nuclei.

FIG. 29. Typical nuclei from an encysted animal showing same decrease in size.

FIG. 30. Typical nuclei from an excysting animal.

CYT, cytoplasm; KS, karyosome; MA, macronucleus; MAL, macronuclear lag; MH, micronuclear halo; MI, micronucleus.

PLATE 9



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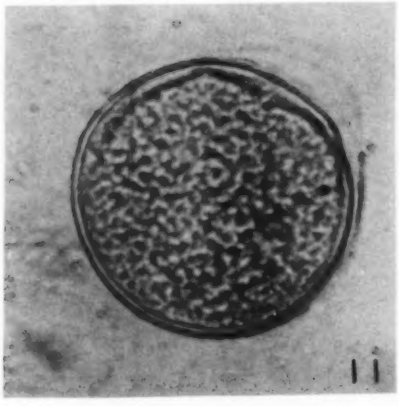
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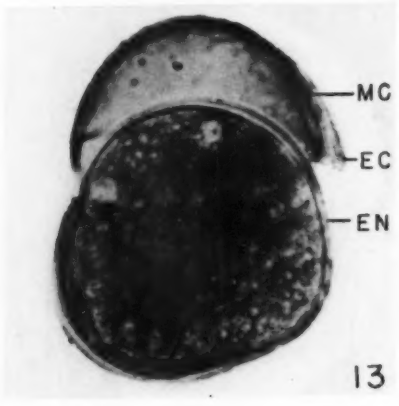

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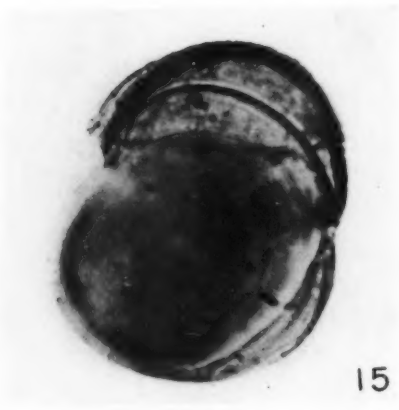
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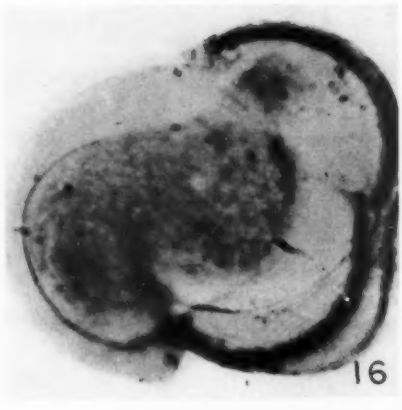
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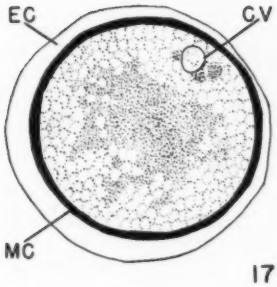
PLATE 11



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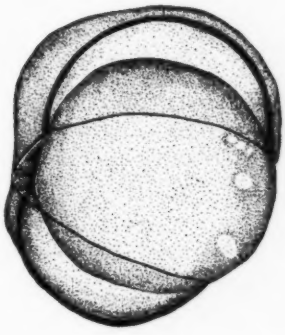

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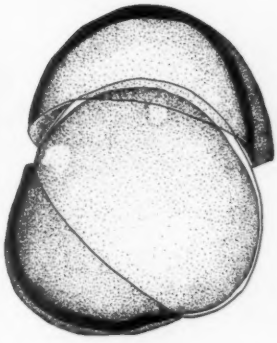

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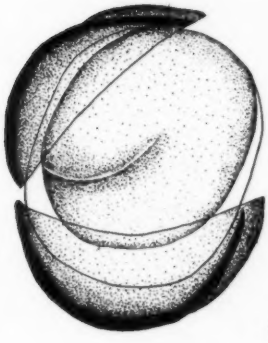

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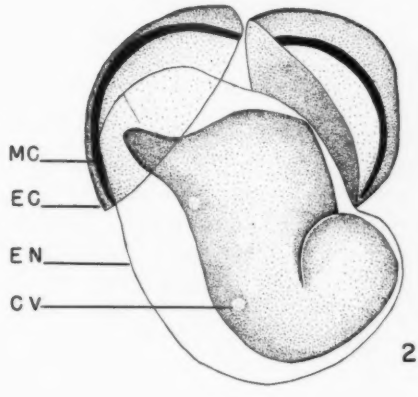

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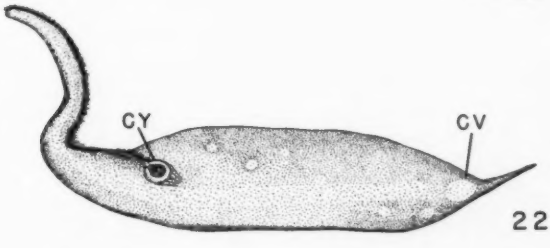

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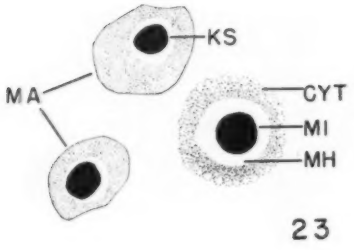
PLATE 12



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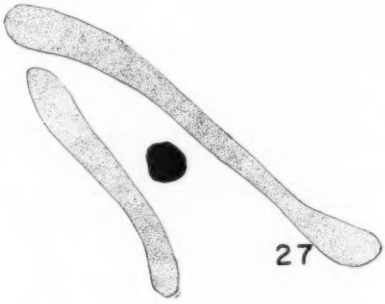

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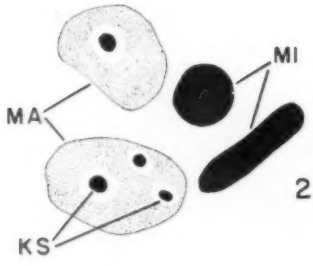

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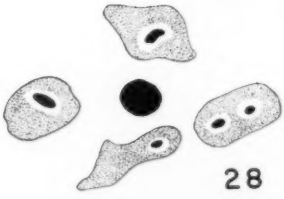

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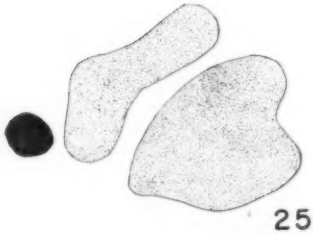

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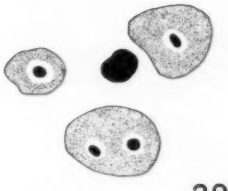

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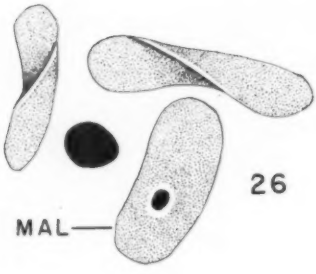

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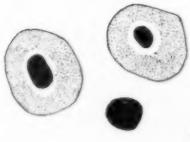

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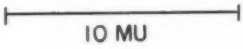

THE RELATION BETWEEN GROWTH SUBSTANCES, CYTOCHEMICAL PROPERTIES OF *TETRAHYMENA GELEII*, AND LESION INDUCTION IN THE CHORIOALLANTOIS

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Plates 13–15

Introduction

It is well established that living cells elaborate materials which facilitate growth. Mast and Pace (1938) maintained that sterile cultures of the colorless flagellate *Chilomonas paramecium* contained an optimum concentration of growth-promoting materials in the first part of the population cycle, while the latter part of the cycle was characterized by a supra-optimum concentration. Kidder (1941) took a different view in regard to limiting factors in population growth. He believed that growing ciliates (*Tetrahymena geleii*) elaborated two substances; one acted as a growth accelerant in young populations while the other inhibited growth in older populations.

Growth-promoting and inhibiting factors with functions similar to those found in protozoan cultures seem to be operative in cellular proliferations of higher animals. Duran-Reynals (1929) demonstrated that testicular extracts applied to the skin in combination with neurovirus enhanced virus action in such a way as to produce lesions that were more hemorrhagic and necrotic than those induced by the virus acting alone. Claude (1935), Sproul *et al.* (1937), and Jobling *et al.* (1937) prepared extracts from Rous sarcoma which produced tumors essentially like the original. An entity which inhibited tumor formation was identified in tumor extracts by Murphy and Sturm (1932a, 1932b) and also by Sittenfield *et al.* (1931). Rohdenburg and Nagy (1937) found that urine from individuals with carcinomata of the uterine fundus or cervix contained materials which facilitated growth in the ciliate *Colpidium campylum*.

There is little available information as to whether protozoan growth-promoting or inhibiting materials facilitate or inhibit growth of vertebrate tissues. The objectives of this study were twofold: (1) to ascertain whether protozoan cultures contain growth-stimulating or both stimulating and inhibiting materials and to study the effects of these materials on physiological processes in vertebrate tissues; and (2) to show the relation between senescence, yield of either or both stimulating and inhibiting materials, phosphatase activity, fat synthesis, and other cytochemical processes in specimens of *Tetrahymena geleii*.

[1] The writer is indebted to Dr. A. M. Elliott for a stock culture of *Tetrahymena geleii E*; to Dr. E. G. Stanley Baker for stock cultures of *Tetrahymena geleii E* and *W*; and to Dr. W. Ray Bryan for Rous sarcomatous tissue.

[2] Supported by a grant-in-aid from the Michigan State College All-College research fund.

MATERIAL AND METHODS

Specimens of *Tetrahymena geleii W* were used exclusively for cytochemical studies. Alkaline and acid phosphatases in young and aged organisms were identified by the methods of Gomori (1941). Feulgen-positive materials were demonstrated by the methods of Bensley and Bensley (1938). Lesions were fixed in Zenker-formol or Petrunkevitch (1933) fixatives and stained with haematoxylin-eosin azure or haematoxylin-eosin. Glycogen was identified with the Feulgen-Bauer reagent subsequent to fixation in alcohol-formol solution. Control slides, immediately after their removal from the fixing solution, were covered with saliva and left for 20 minutes before immersion in the staining reagent.

Specimens of *Tetrahymena geleii E* and pedigreed White Leghorn hatching eggs were used exclusively in the portion of this study concerned with lesion induction. All organisms used for experimentation were washed in three changes of normal saline by the method of Mast and Fennell (1938), and then they were suspended in normal saline and left for 2–4 hours. Organisms were inactivated in a water bath (at 50 degrees) and 0.05-cc. portions (1–10 dilution) were used for inoculation of the chorioallantois of 14-day embryos by the artificial air-sac method of Burnet (1936). Some suspensions were prepared from young cultures; others from aged cultures; and the rest from aged cultures contaminated with a slow-growing mold. A 1–4 saline dilution of Rous sarcoma was used in some experiments for inoculation of the chorioallantois.

Trichloracetic acid soluble materials were prepared from 0.4 cc. of organisms suspended in 1 cc. of saline. Trichloracetic acid (4 cc. of 5 percent) was added to the suspension and then it was left overnight in the ice box. The suspension was centrifuged and the supernatant extract was removed, frozen, evaporated to dryness with a vacuum pump, and resuspended in 1.5 cc. of normal saline. This suspension, in 0.07-cc. portions was used for the inoculation of the chorioallantois of 14-day embryos.

Alcohol-soluble materials were prepared from 0.15 cc. of *Tetrahymena* collected from aged cultures. The volume was made up to 1 cc. with saline and then 2 cc. of absolute alcohol and 3 cc. of chloroform were added. The mixture was vigorously shaken and then centrifuged. A 3-phase system was thus established in the centrifuge tube; chloroform at the bottom, proteins in the center, and alcohol-soluble materials on top. The latter were removed, frozen, evaporated to dryness with a vacuum pump, and then the residue was resuspended in 1 cc. of saline, and 0.07-cc. portions of the suspension were used for inoculation of the chorioallantois of 14-day embryos.

RESULTS

1. *Cytochemical properties of Tetrahymena geleii*

Specimens of young and aged *T. geleii W* were used exclusively for cytochemical studies. Organisms described as aged specimens in subsequent pages were obtained from cultures 10–30 days after seeding and individuals referred to as young specimens were removed from cultures 3–10 days subsequent to seeding. Control slides were stained for comparison with slides treated with glycerophosphate medium.

Alkaline and acid phosphatase. A comparison of Figure 1 with Figure 2 shows that the concentration of alkaline phosphatase is relatively lower in young cells than it is in aged cells. The latter exhibit phosphatase activity in all regions of the cell, while in the former, phosphatase is restricted to the distal ends of the cells. The phosphatase reaction in some of the aged cells may depend on naturally occurring calcium salts, e.g., 37.94 per cent of the control organisms from an aged culture exhibited black precipitates in the distal ends of the cells. On the other hand, 71.88 per cent of the organisms from this same culture when treated with glycerophosphate gave a positive reaction for enzymatic activity.

After an examination of Figure 4 it is evident that young cells exhibit a few acid phosphatase positive granules. In aged specimens acid phosphatase increases (Fig. 5), but the total increase is less than it was in the case of alkaline phosphatase (Fig. 1). It is also evident in Figure 5, that the nuclei of aged cells exhibit a weak positive reaction for acid phosphatase.

Fat. A comparison of Figure 3 with Figure 6 shows that the volume of fat in some aged cells is several times greater than it is in young cells. In the latter, fat positive granules (osmic-chromic acid stain) were restricted to the apical end of the cells, while on the other hand, fat positive granules extended from the apical to the distal end of aged cells. There is, however, variation in both volume and type of fat found in individuals from an aged culture—e.g., of 373 individuals stained with osmic-chromic acid solution, 328 exhibited fat granules occupying from $\frac{1}{3}$ to $\frac{1}{2}$ of the cell, and in the rest, fat-positive granules filled the entire cell. In other aged organisms, stained with dilute nile-blue sulfate, about $\frac{2}{3}$ of the granules gave a positive reaction for neutral fat and the remaining third was positive for fatty acids. Fat granules, subsequent to staining with Sudan IV, were red, dust-like particles restricted to the anterior $\frac{2}{3}$ of the cell, which after standing, coalesced to form larger rose-red units.

Nucleic Acid. The Feulgen reaction was unpredictable and difficult to demonstrate in specimens of *T. geleii*. Specimens from 2 of 17 cultures showed Feulgen-positive granules after hydrolyzation for 4 and 10 minutes, respectively. Hydrolyzation for the shorter time demonstrated an abundance of precisely stained bluish-purple granules within the nuclei (Fig. 9). However, many nuclei in the vicinity of the reactant ones were stained yellowish brown and others did not show any observable reaction with the reagent. Hydrolyzation for the longer period demonstrated an abundance of granules in the cytoplasm, which were spherical and variable in number (Fig. 8). Figure 7 shows an aged cell, with numerous cytoplasmic basophilic vesicles, obtained after iron-haematoxylin stain, subsequent to hydrolyzation in N/HCl for 14 minutes. Hydrolyzation for 4 or 10 minutes did not differentiate these vesicles so precisely.

Feulgen-positive[3] nuclear materials exhibited the following optical characteristics: (1) refractive index of about 1.65; (2) anisotropic; (3) negative elongation; and (4) parallel extinction. Characteristics 3 and 4 suggest negative uniaxillarity.

Glycogen. Specimens used in this series of experiments were obtained from a

[3] I am indebted to Dr. B. T. Sandefur for the optical analyses of the Feulgen-positive nuclear materials.

sterile tryptone solution in which organisms were abundant (72 hours subsequent to seeding). One-tenth cc. of specimens was concentrated in a 15-cc. centrifuge tube and then they were resuspended in 1 cc. of sterile tryptone. Ninety-six per cent of the organisms, 24 hours after resuspension in tryptone solution, showed an abundance of granular glycogen uniformly distributed throughout the cytoplasm of the cells. Both frequency of occurrence and volume of glycogen seemed to be closely related with the length of time organisms were left in tryptone solution. About 48 hours after resuspension 55 per cent of the organisms exhibited granular glycogen, and at 72 hours the number had decreased to 27 per cent. Control slides were negative for glycogen. The volume of fat and alkaline phosphatase was affected to a lesser degree by crowding.

2. *Lesion induction in the chorioallantois*

Frequency of lesion induction. It is evident in Table 1 that the mean percentage of lesions (57.7) induced by saline suspensions of aged organisms was significantly higher than the percentage of lesions (0.2) induced by suspensions made with young cultures. Aged cultures used for experimentation in the summer of 1946 differed from those used in the summer of 1947. The former contained a slow-growing undetermined mold which greatly facilitated population growth. In lesions (4 of 16) induced by inoculums made with mold-contaminated cultures there was an abundance of intranuclear and cytoplasmic inclusions in cells of the hyperplastic epithelium. It was found during the course of this study that there was a predisposition toward variability in volume of fat found in specimens of aged *T. geleii* (Fig. 6). The writer while assisting Mast (1938) with the study of fat digestion found that specimens of *Colpidium striatum* from mold-contaminated cultures exhibited an abundant number of organisms with an above-average fat and fatty acid content. Aged organisms obtained from mold- and bacteria-free cultures during the summer of 1947 exhibited less fat and they induced lesions in which epithelial inclusions were absent.

It is also evident in Table 1 that two types of lesions were induced by the inoculums used in this investigation, sc.: (1) gray thickened areas in the chorio-allantois and (2) umbilicated lesions. Saline suspensions of aged organisms induced both types of lesions. Inoculums prepared from cells obtained from young cultures, trichloracetic acid materials, lesion and liver mince, alcohol-soluble materials and supernatant extract induced Type 1 lesion. The smallest number of lesions was induced by suspensions of young cells (0.2 per cent) and the largest number was induced by alcohol-soluble materials (65 per cent).

Aged specimens of *Tetrahymena* suspended in saline induced hemorrhages at the inoculation site; these observations suggested that a rather close correlation might exist between hemorrhaging of the chorioallantois and induction of umbilicated lesions. Such a hypothesis is refuted by Experiments 4 and 5. Trichloracetic acid-soluble materials and alcohol-soluble materials induced relatively large hemorrhages yet the chorioallantoic lesions that developed in the hemorrhagic area were relatively insignificant gray thickened areas. Some of the lesions induced by the acid-soluble materials exhibited one unique feature in that the

TABLE 1

Chorioallantoic lesions induced by specimens of Tetrahymena *suspended in normal saline and by various types of extracts*

In Experiment No. 1, 0.05 cc. of a 1–10 dilution made with aged cultures; No. 2, 0.05 cc. of a 1–10 dilution made with young cultures; No. 3, 0.1 cc. of supernatant extract from a 1–10 dilution of organisms from aged cultures; No. 4, 0.07 cc. of trichloracetic acid soluble materials resuspended in saline; No. 5, 0.05 cc. of saline suspended alcohol-chloroform soluble materials of *Tetrahymena* from aged cultures; No. 6, 0.05 cc. of grossly normal embryonic liver in saline (1–4 dilution); No. 7, 0.05 cc. of lesion and liver mince (equal quantities of each) suspended in normal saline (1–4 dilution); No. 8, 0.05 cc. of a 1–4 dilution of Rous sarcoma; No. 9, 0.05 cc. of an alcohol-chloroform extract (alcoholic fraction) suspended in saline.

EXP. NO.	TYPE OF INOCULUM USED	NO. OF EMBRYOS INJECTED	PER CENT SURVIVAL OF EMBRYOS	PER CENT DEVELOPING LESIONS	PRINCIPAL TYPES OF LESIONS	TISSUE REACTION	CELLULAR REACTIONS	SPECIAL FEATURES
1	*Tetrahymena* from aged cultures (1–10 dilution)	52	78.9	57.7	1. Gray thickened area on chorioallantois 2. Umbilicated lesions (diam. from 5–10 mm.)	Moderate to pronounced hyperplasia of all chorioallantoic tissue elements	Intranuclear and cytoplasmic inclusions	1. Hemorrhages at most injection sites. 2. Nests of epithelial cells
2	*Tetrahymena* from young cultures	44	99.3	0.2	Gray thickened areas on chorioallantois	Moderate hyperplasia of all chorioallantoic tissue elements	Inclusions absent	
3	Supernatant extract of aged *Tetrahymena*	21	33.4	14.3	Gray thickened areas on chorioallantois	" "	Inclusions absent	2 of embryos smaller than normal
4	Trichloracetic acid soluble materials of *Tetrahymena*	14	42.9	21.4	Gray thickened area on chorioallantois	" "	Inclusions absent	Hemorrhages at injection sites
5	Alcohol-chloroform soluble materials from aged *Tetrahymena*	20	85.0	65.0	Gray thickened areas on chorioallantois	" "	Inclusions absent	Hemorrhages at most injection sites
6	Grossly normal embryonic liver	21	90.5	9.5	Gray thickened areas on chorioallantois	" "	Inclusions absent	
7	Lesion and liver mince	6	83.4	16.6	Gray thickened areas on chorioallantois	" "	Inclusions absent	
8	Rous sarcoma mince	14	85.7	71.4	Gray thickened areas on chorioallantois	Moderate to pronounced hyperplasia of mesodermal elements	Inclusions absent	
9	Alcohol-chloroform soluble materials from Rous sarcoma	15	86.7	60.0	Gray thickened areas on chorioallantois	Moderate hyperplasia of mesodermal elements	Inclusions absent	

center of each lesion showed an insoluble precipitate completely invested by hypertrophied mesodermal tissue elements.

Umbilicated lesions. The diameter and thickness of the lesion varied (5–15 mm. and 3–5 mm., respectively); they were sharply delineated from the surrounding tissue and the thinner peripheral portions formed a closed circle with an elevated central portion. In the central region of such lesions there was a distinct umbilicus; others of these lesions were more plaque-like. The rim of some umbilici frequently exhibited pileus-like or foliate extensions of epithelium (Fig. 13). Epithelium in the vicinity of the umbilicus exhibited significant hyperplasia and necrotic areas were of frequent occurrence. In areas where stratification (hyperplasia) was pronounced, epithelial cells adjacent to the mesoderm were closely packed and compressed while the more distally located cells were larger and of variable size. Epithelial cells nearest to the embryo (endoderm) frequently exhibited hyperplasia but to a lesser degree.

Mesodermal elements exhibited pronounced hyperplasia (Fig. 13). In some areas there were abundant amounts of fibrous material while in other areas there were increased numbers of fibroblasts. It is evident in Figure 13 that fibroblasts immediately adjacent to the hypertrophied epithelium were packed to form a relatively dense layer of cells.

Gray thickened areas in the chorioallantois. The principal difference between this lesion and the umbilicated lesion was the degree of hyperplasia in the various tissue elements. A comparison of Figures 10, 12, and 14 with Figure 13 will show that the epithelium in the former exhibited less hyperplasia than it did in the latter. Ectodermal epithelium frequently exhibited spaces of various sizes. In some lesions the epithelium consisted of a single layer of cells and hyperplasia was restricted to the mesodermal elements (Fig. 14). Nests of epithelial cells investing fibrous materials were frequently found in the mesoderm (Figs. 12 and 27). In some areas there were excessive amounts of fibrous materials and in other areas there were increased numbers of fibroblasts (Figs. 12 and 14). One of the lesions induced by alcohol-soluble materials consisted of mixed masses of fibrils and epithelial cells (Fig. 11).

The mesodermal elements of the gray thickened areas had one characteristic in common with umbilicated lesions. Fibroblasts in many areas adjacent to the hyperplastic epithelium exhibited a pronounced hyperplasia and as a consequence the fibroblasts were packed to form closely compacted masses of cells. In other areas further removed from the hyperplastic epithelium, the fibroblasts were more loosely arranged and an interspersion of connective tissue fibers was of frequent occurrence.

Rous sarcoma lesions. Approximately seventy-one per cent (10 of 14) of the chorioallantoic membranes injected with Rous sarcomatous tissue developed disc-like lesions of variable size (diameter, 5–20 mm.; thickness, 2–4 mm.). Alcohol soluble materials of Rous sarcoma (alcohol-chloroform extraction) resuspended in saline induced lesions in 9 of 15 embryos. Lesions induced by these methods showed a strong resemblance to the gray thickened chorioallantoic areas induced by suspensions of aged *Tetrahymena*.

A microscopic comparison of *T. gelei*-induced lesions with sarcomatous lesions revealed both likenesses and differences in their structure and appearance. The former showed cellular areas of fibroblast-like cells which appeared to be essentially similar to mesodermal elements in Rous sarcoma. The size of both umbilicated and gray thickened chorioallantoic lesions was determined to a great degree by size and frequency of epithelial nests and proliferation of surface epithelium. On the other hand, size of Rous sarcoma lesions was determined, in a large measure, by proliferation of mesodermal elements.

Inclusions. Organisms from aged, mold-contaminated cultures suspended in saline produced well defined intranuclear and cytoplasmic inclusions within the epithelium of 4 of the 16 chorioallantoic lesions examined. The intranuclear inclusion with the highest frequency of occurrence was the multiple type which consisted of eosinophilic granules of various sizes (Figs. 16, 17, and 18).

Figure 17 shows well defined inclusions within the nucleus of a cell that appears to be essentially normal. Variations in morphological features of inclusion-bearing nuclei were fairly common. Figure 16 shows an inclusion-bearing epithelial cell with a relatively compact mass of basi-chromatin in the lower fifth of the nucleus and a uniformly distributed mass of eosinophilic material in the upper $\frac{4}{5}$ of the nucleus. Further, it is evident in Figure 18 that four large inclusions were in the nuclear area of the cell and that the major portion of the nuclear membrane was absent. In other epithelial cells with intranuclear inclusions, the nuclear membrane was shrunken and strands of protoplasm extended from the nuclear membrane to the cell membrane (Fig. 19). Emargination of chromatin and the presence of halos around inclusions were likewise observed (Fig. 20). Another interesting type of inclusion was found in the flattened epithelial cells immediately adjacent to the mesoderm. A few of the cells in this area frequently exhibited from 1–5 intranuclear inclusions arranged to form a linear series. Each inclusion was separated from adjacent inclusions by a membrane-like structure (Figs. 22 and 23), and, as a consequence, the nucleus appeared to be segmented. One nucleus showed such segmentation yet only one inclusion was identified within the nucleus (Fig. 24). Figures 25 and 26 show characteristic types of cytoplasmic inclusions found within epithelial cells. The former appeared to be invested by a halo, but in the latter the halo was absent.

DISCUSSION

Wislocki and Dempsey (1945) maintain that in vertebrate tissues alkaline phosphatase plays an important role in the synthesis of glycogen by dephosphorylation of hexose-phosphate. Results presented in this report revealed relatively abundant amounts of glycogen in specimens taken from young cultures. As the age of the culture increased the volume of glycogen decreased, and the volume of fat increased. In the intervening period between glycogen loss and the appearance of neutral fat, the volume of alkaline phosphatase showed a pronounced increase. Acid phosphatase increased to a lesser degree. Since the spatial relationships between fat and alkaline phosphatase are essentially the same in aged organisms, it is interesting to speculate on the role of alkaline phosphatase

in fat synthesis. Baldwin (1948) in a discussion of fermentation states that under certain conditions phosphoglycerol arises from carbohydrates. This material is hydrolyzed by yeast phosphatase to form glycerol, CO_2, and an additional compound. Observations made in this study revealed relatively large amounts of fatty acids in specimens of *Tetrahymena*. If this hypothesis is carried farther, and it is assumed that glycerol does exist in *Tetrahymena*, the component parts of neutral fats, i.e., glycerol and fatty acids, are available for synthesis of fats. This view concerning fat synthesis is interesting, but as yet too little is known about the specificity of the various histochemical reactions in the protozoa to reach any definite conclusion in regard to the role of alkaline phosphatase in fat synthesis.

Suspensions of senescent specimens of *Tetrahymena*, which induced pronounced proliferation in chorioallantoic tissues, exhibited abundant amounts of neutral fats, fatty acids, nucleoproteins, calcium salts, and pronounced phosphatase activity. Fractionated portions of such cells, i.e., supernatant extracts, and trichloracetic acid-soluble materials, were much less effective tissue stimulants. There was evidence of a differential effect of these materials, i.e., alcohol-chloroform extracts induced lesions in 65 per cent of the chorioallantoic membranes, but the degree of tissue proliferation was less than it was when intact cells were used. These results show that the chemical constituents of senescent cells exert an additive effect, and under the conditions found in the chorioallantois of 14-day embryos, tissue proliferation is increased to a maximum.

Entities that are responsible for tissue stimulation in Rous sarcomatous tissues are similar to tissue stimulants in senescent *Tetrahymena* in several respects: (1) the intact Rous sarcomatous cells induced the most pronounced proliferation of chorioallantoic tissues; and (2) alcohol-chloroform extracted materials contain an active tissue stimulant. The question immediately arises as to the role of cellular senescence in carcinogenesis. Irritants (croton oil, etc.) when applied to the skin for prolonged periods induce carcinogenetic changes in normal tissues. Gross appearance of any irritated area suggests that among the underlying causes for this condition are necrosis and the build-up of abnormal amounts of normally occurring cellular materials. Release of these materials into adjacent tissues exposes "normal" cells in senescent areas to abnormal concentrations of growth stimulating materials, which not only facilitate tissue proliferations, but also induce important side effects, i.e., the capillary bed is impaired and hemorrhagic areas become evident. Under such conditions, tissue proliferations are maintained at high levels, senescence is enhanced and release of abnormal quantities of growth-stimulating materials into adjacent areas by necrosis repeats the cycle of events which ultimately ends in carcinogenesis.

Senescence in vertebrate tissues is made more complex by other factors. Each species as well as each individual within a species varies in regard to its ability to withstand tissue senescence, i.e., genetically resistant tissues withstand senescence to a greater degree than susceptible tissues. Nevertheless, in some malignancies an introduction of a sufficiently high concentration of growth-stimulating

materials and senescent cells into resistant animals induces a chain of events in the tissues that may be repeated over and over again.

A reference has already been made to the induction of intranuclear and cytoplasmic inclusions in epithelial tissues. Some of the observations cited in an earlier section indicated that the volume of fat and phosphatase activity in specimens of *Tetrahymena* from mold-contaminated cultures was greater than it was in specimens from mold- and bacteria-free cultures. A positive phosphatase reaction was obtained in many aged cells although incubation of specimens in glycerophosphate medium was omitted. This reaction depends on the presence of preformed calcium salts. If such salts are basic in reaction, they can combine with fat to form calcium soaps which are very insoluble in water. The basic nature of intranuclear inclusions is well established, i.e., they are stained with acid dyes. If the intranuclear inclusions were soaps, how did they get into the epithelial tissues of the chorioallantois? Since both *Tetrahymena* and epithelial tissues are highly specialized, senescent changes in the former might closely parallel those taking place in the latter. If this is true, there is reason to suspect that any agent, i.e., salts of heavy metals or senescent cells, might induce senescent changes in normal vertebrate tissues which were characterized by the development of intranuclear inclusions.

SUMMARY

1. There is a direct relation between age and concentration of neutral fat, fatty acids, Feulgen-positive materials, alkaline phosphatase and acid phosphatase in specimens of *T. geleii*.

2. Saline suspensions of *Tetrahymena* from aged, mold-contaminated cultures; saline suspensions of aged, bacteria- and mold-free organisms; saline suspensions of young organisms and various extracts were used for inoculation of the chorioallantoic membranes of 14-day chicken embryos.

3. The inoculums used induced two types of lesions, i.e., (1) gray thickened areas in the chorioallantois and (2) umbilicated lesions.

4. Of the chorioallantoic membranes that received saline-suspensions of *Tetrahymena* from aged cultures 57.7 per cent developed either Type 1 or Type 2 lesions. Umbilicated lesions (Type 2) induced by inoculums prepared from aged cultures contaminated with a mold differed from umbilicated lesions induced by inoculums made with aged, bacteria-free cultures in that the former exhibited abundant intranuclear and cytoplasmic inclusions. Alcohol-chloroform extracted materials induced lesions in 65.0 per cent of the chorioallantoic membranes, but the lesions induced in this manner did not exhibit inclusions.

5. Type 1 lesions were induced by saline-suspended *Tetrahymena* from young and aged cultures, alcohol-chloroform extracted materials, trichloracetic soluble materials, lesion-liver mince, supernatant extracts and grossly normal embryonic liver.

6. Of the chorioallantoic membranes injected with Rous sarcomatous tissue 71.4 per cent developed lesions. Sixty per cent of the chorioallantoic membranes injected with alcohol-chloroform materials developed lesions.

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EXPLANATION OF PLATES

PLATE 13

Cytochemical reactions in specimens of young and aged *Tetrahymena geleii W*. Figures 1–8, incl. × 680; 9, × 1020. Photomicrographs, courtesy of Dr. R. F. Langham.

FIG. 1. Alkaline phosphatase and preformed phosphates in aged cells and fragments of cells.

FIG. 2. Alkaline phosphatase in young cells.

FIG. 3. Young cells stained to show fat with osmic-chromic-acid reagent.

FIG. 4. Acid phosphatase in young cells.

FIG. 5. Acid phosphatase in aged cells.

FIG. 6. Aged cells stained to show fat with osmic-chromic acid reagent.

FIG. 7. Basophilic cytoplasmic bodies in aged cells. Iron-haemotoxylin stain following hydrolyzation in N/HCl for 14 minutes.

FIG. 8. Feulgen-positive cytoplasmic bodies in aged cells.

FIG. 9. Feulgen-positive nuclear granules in aged cells.

PLATE 14

Photomicrographs of chorioallantoic lesions induced by saline suspensions of *Tetrahymena* and by extracts of *Tetrahymena*. EE, ectodermal epithelium; M, mesoderm. Fixed in Petrunkevitch fixative; Figures 11, 13 and 15 stained with haematoxylin-eosin-azure; 10, 12 and 14 stained with haemotoxylin-eosin. Lesions shown in Figures 10, 12, 13, 14 and 15 induced by saline-suspensions of aged organisms; 11, induced by saline suspension of alcohol-chloroform soluble materials. Figures 10, 11, 12, 13 and 14, × 82; 15, × 1050: Bausch and Lomb × 90 apochromatic objective; compensating ocular, × 10. Photomicrographs, courtesy of Professor R. L. Sweet.

FIG. 10. Cross-section of a gray thickened area in chorioallantois showing moderately hyperplastic epithelium resting on a compact layer of fibroblasts.

FIG. 11. Frontal-section of a gray thickened chorioallantoic area showing hyperplasia of the epithelium.

FIG. 12. Cross-section of a gray thickened chorioallantoic area showing nests of cells investing fibrous material.

FIG. 13. Cross-section of umbilicated lesion showing a pronounced hyperplasia of epithelium and mesodermal elements.

FIG. 14. Cross-section of a gray thickened area in the chorioallantois with relatively little epithelial hyperplasia and moderate hyperplasia of the mesoderm.

FIG. 15. Epithelium of Figure 13 showing an area where inclusions are abundant.

PLATE 15

Camera lucida drawings of epithelial cells from chorioallantoic lesions showing various types of intranuclear and cytoplasmic inclusions. All embryos injected with a mold-contaminated culture of aged *Tetrahymena* suspended in saline. All tissues were fixed in Petrunkevitch fixative. Cells in Figures 16, 17, 20, 21 and 27 were stained with haematoxylin-eosin-azure and cells in 18, 19, 22, 23, 24, 25 and 26 were stained with haematoxylin-eosin. Figures 16–26, × 1770; Figure 27, × 1070.

FIG. 16. Epithelial cell showing multiple inclusions with basichromatin concentrated in one end of nucleus; oxychromatin uniformly distributed in rest of nucleus.

FIG. 17. Epithelial cell showing multiple inclusions in nucleus.

FIG. 18. Epithelial cell showing 3 relatively large multiple inclusions; nuclear membrane evident on one surface of inclusions.

FIG. 19. Epithelial cell showing shrunken nucleus with various sizes of multiple inclusions.

FIG. 20. Epithelial cell showing multiple inclusions with emarginated basichromatin.

FIG. 21. Epithelial cell with emarginated chromatin and a single intranuclear inclusion.

FIG. 22. Epithelial cell from a region adjacent to mesoderm showing a segmented-like nucleus with an inclusion in each segment.

FIG. 23. Epithelial cell showing a segmented-like nucleus with an inclusion in each segment.

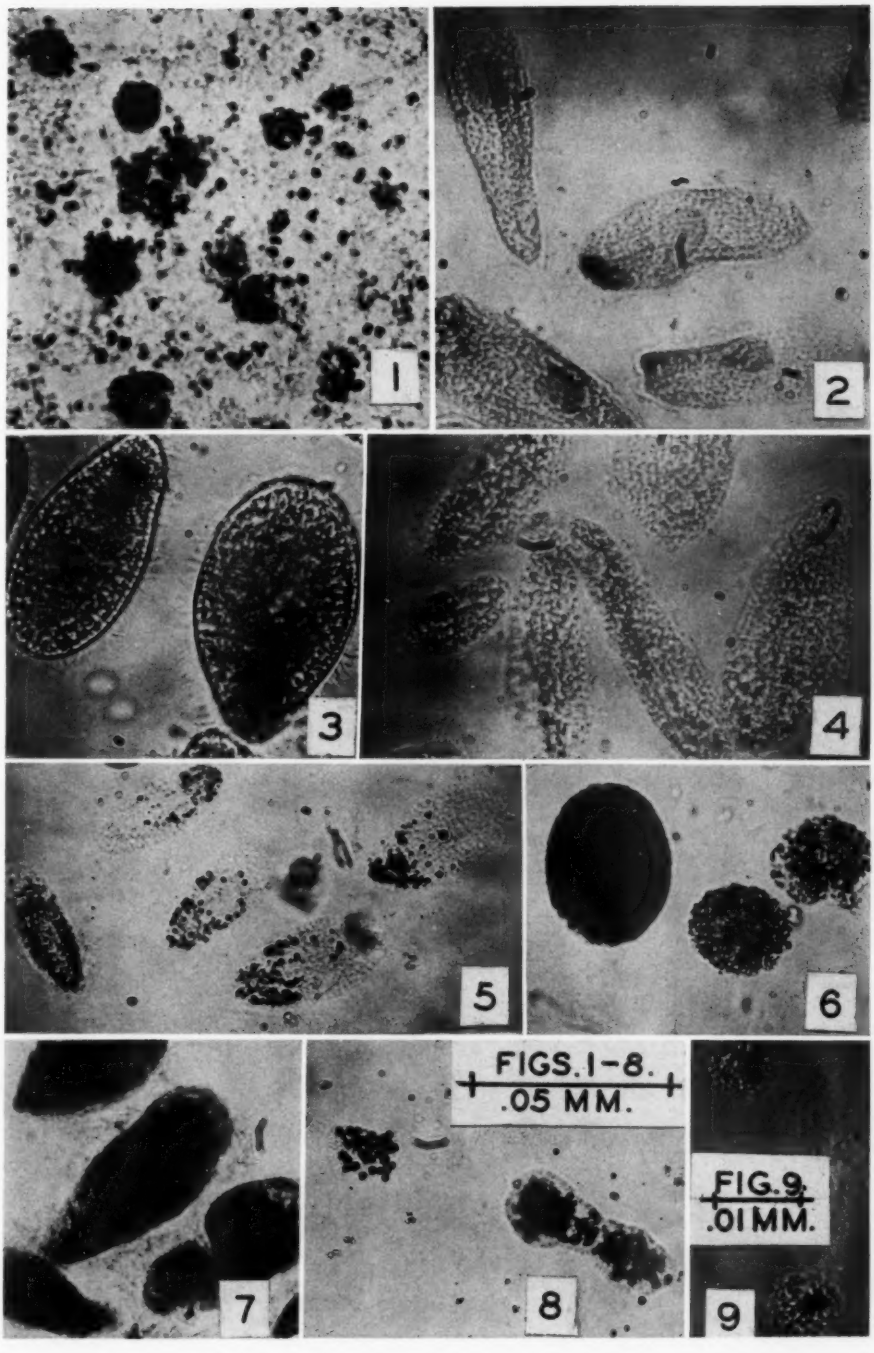
FIG. 24. Epithelial cell showing a segmented-like nucleus with one division showing an intranuclear inclusion.

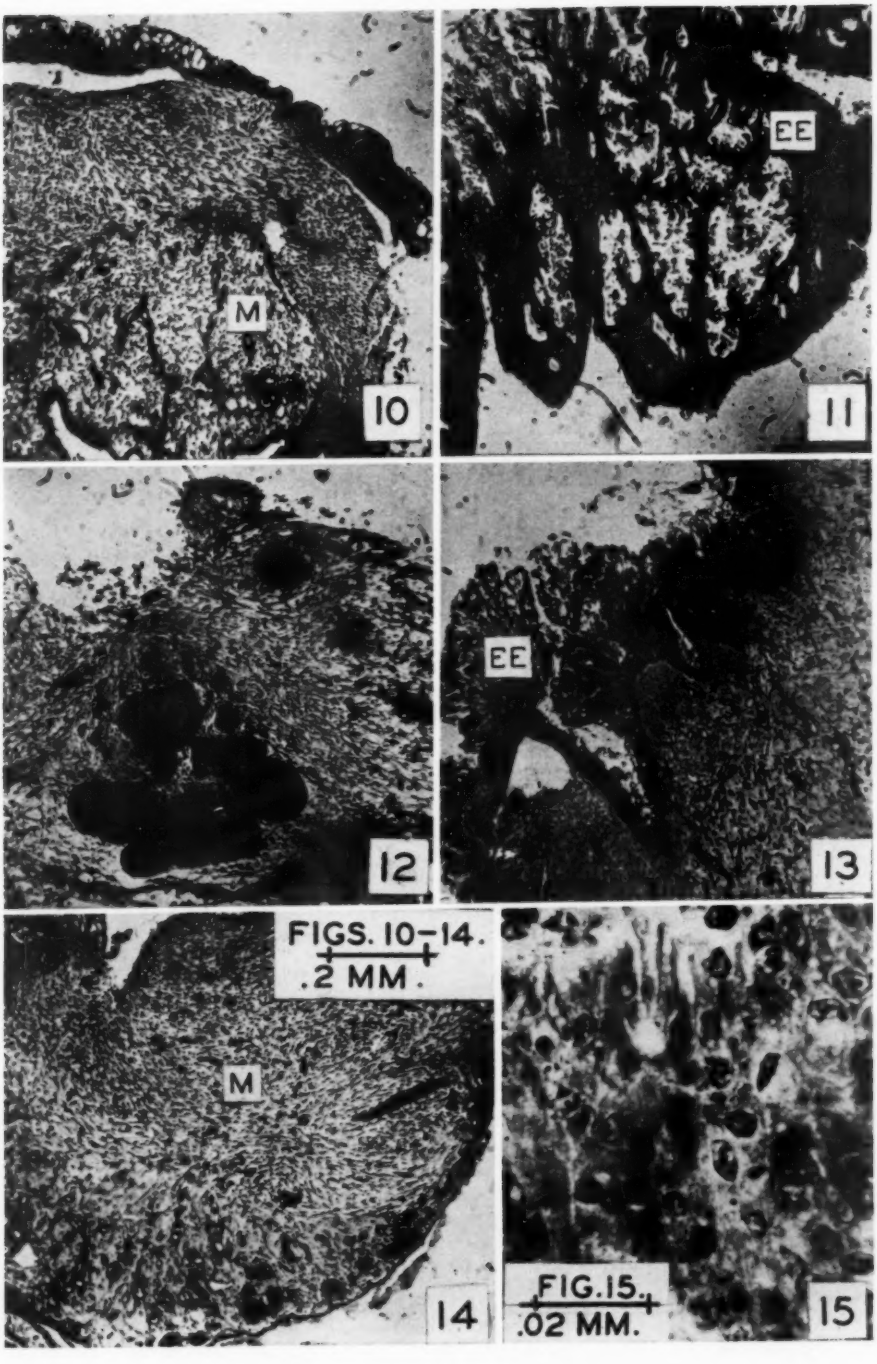
FIG. 25. Epithelial cell showing multiple cytoplasmic inclusions surrounded by a halo.

FIG. 26. Epithelial cell showing cytoplasmic inclusions.

FIG. 27. Nests of epithelial cells surrounding fibrous material.

PLATE 13




M
10
EE
11
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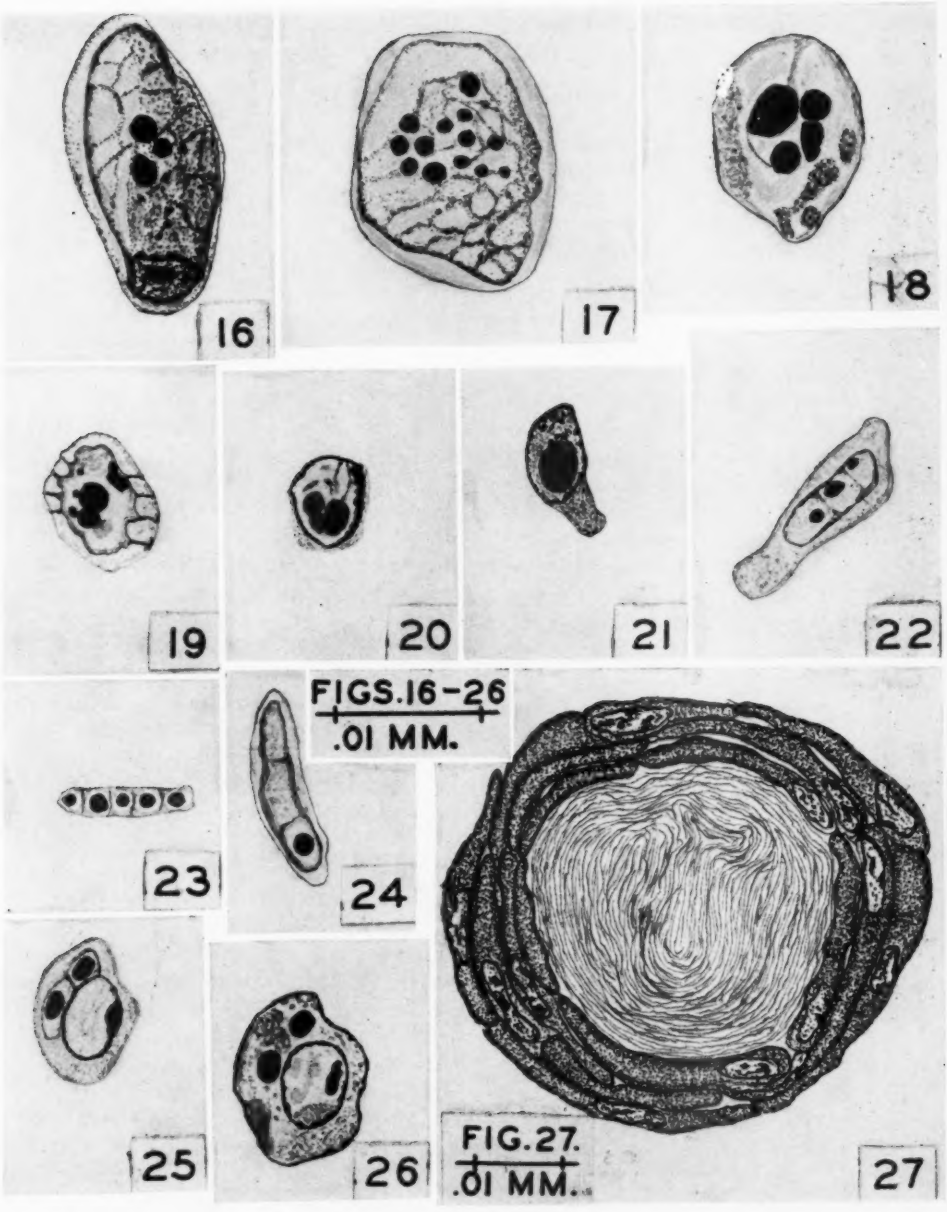
13
FIGS. 10–14.
.2 MM.
M
14
FIG. 15.
.02 MM.
15

PLATE 15



SOME NEW SPECIES OF BEES FROM THE EASTERN UNITED STATES[1]

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Plate 16

A faunistic study of the bees occurring in the eastern United States has resulted in the discovery of a considerable number of species which appear to be undescribed. Some of these were recognized as new species by previous workers, and were given names, but these names and descriptions were never published.

Descriptive work on three of the more extensive genera has been completed. The previously unnamed species of two of these genera, *Colletes* and *Hylaeus*, are described in the following pages, together with one of the species of *Andrena*. This last description is included to make possible the use of the name in some studies of its biology. Other new species of *Andrena* are not included, to avoid unduly lengthening this paper. This genus is a very extensive one and includes many species which are in need of description. These will be treated in future papers or in a proposed manual of the bee species of this area.

Valuable assistance has been received from Dr. P. H. Timberlake, of the Citrus Experiment Station, Riverside, California, who has examined these materials, compared them with the allied western species, and concurred in the opinion that they are not conspecific with any of the described species, eastern or western. Certain of his comments will be quoted following the descriptions.

I wish to acknowledge also the cooperation of Mr. William P. Stephen at the University of Kansas with whom I have corresponded concerning the status of certain of the species of *Colletes*.

Colletes brimleyi new species

Female—Length 8–10 mm.; length and breadth of face subequal; eyes slightly convergent below; malar space about one-third as long as broad; facial foveae moderately shining, rounded above; clypeus shining between rather close and fine punctures, these somewhat more close and fine on sides of face and on front below ocelli, minute on vertex and cheeks; hind margin of vertex indefinite, rounded, separated from lateral ocelli by somewhat more than their diameter; basal segment of flagellum hardly longer than pedicel, second very slightly shorter, middle segments about as long as broad; pubescence pale ochraceous on head and thorax, becoming deeper ochraceous or nearly fulvous on dorsum of thorax; lateral angles of prothorax short, acute but not spine-like; metapleural

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protuberance not carinate; front coxae simple; mid tibiae angulate beneath toward base; hind metatarsi slightly more than three times longer than broad (3.4:1); spurs yellowish; tegulae yellowish-ferruginous; wings hyaline or nearly, nervures and stigma ferruginous; second and third submarginal cells subequal, second receiving first recurrent near middle, third receiving second recurrent one-third or less from tip; punctures moderately coarse and deep on thorax, rather close on anterior half of mesonotum and most of scutellum, more sparse in center of mesonotum and along anterior margin of scutellum, more coarse and close on pleura, which are subrugose; posterior face of propodeum shallowly rugose, the triangle smooth below, with a few irregular striae above, upper face composed of a transverse series of shining pits, posterior margin carinate; abdominal terga smooth and shining, with minute punctures, well separated on basal segment, quite close on the others; discs of terga with very obscure short pile which seems to be largely pale, the more apical segments with scattered erect and rather long pale hairs; apical fasciae entire, whitish or ochraceous; sixth tergum dark in its general aspect, but with some long pale hairs.

Male—Length 8–10 mm.; length and breadth of face subequal; eyes slightly convergent below; length of malar space about half its breadth; facial foveae small but quite distinct; distance of lateral ocelli from edge of vertex greater than their diameter; edge of vertex less rounded than in female; basal segment of flagellum subequal to pedicel, second and following segments twice as long, flagellum ferruginous to piceous beneath; punctures fine and quite close on clypeus beneath the long pubescence; labrum highly polished, with a shallow median impression; front below ocelli finely rugose, vertex very finely so, cheeks more shining, striately punctate; pubescence pale ochraceous, becoming deeper ochraceous or fulvous on dorsum of thorax, long and dense on face, clypeus and cheeks, thinner on vertex; lateral angles of prothorax short, acute, not spine-like; metapleural protuberance not carinate; all metatarsi slender, yellowish-ferruginous; spurs yellowish; tegulae dark in center, yellowish-ferruginous around margin; wings hyaline or nearly, nervures and stigma ferruginous, second and third submarginal cells subequal, recurrent nervures as in female; punctures quite coarse, deep and close on anterior half of mesonotum, but the anterior margin medially shining and impunctate, center of posterior half polished and sparsely punctate; scutellum with quite coarse crowded punctures, but anterior margin smooth and shining; metanotum more finely rugose; pleura coarsely rugose except just above mid coxae where the punctures are distinct and rather sparse; lateral and posterior faces of propodeum striately rugose, triangle smooth and shining below, more striate above, dorsal face a series of rectangular shining pits, posterior margin carinate; abdominal terga shining, distinctly though very finely punctate, punctures well separated on all segments except 5-7, hind margins of terga obscurely reddened, discs with very short obscure pile of uncertain color, the more apical segments with scattered, long, erect, pale hairs; wings of seventh sternum very broad, apical margin of each broadly and evenly incurved, the resulting lobes subequal, discs minutely hairy except along outer side near middle; gonostyli slender apically, rather long pubescent, slightly exceeded by the penis

valves which are quite broad basally; cuspis of volsella slender, slightly longer than the rounded digitus.

Holotype: Female, Fort Macon, N. C., May 11, 1936 (C. S. Brimley). Allotype, topotypical [both N. C. Dept. Agr.]. Paratypes: 2 ♀ ♀, 19 ♂ ♂, topotypical (C. S. Brimley) [N. C. Dept. Agr.]; 6 ♀ ♀, 28 ♂ ♂, Inverness, Fla., 1892 (Charles Robertson) [Ill. Nat. Hist. Surv.]; 1 ♀, Miami, Fla., Apr. 11, 1923 [Am. Mus. F.4666B]; 1 ♀, St. Marys, Ga., Mar. 6–Apr. 18, 1896 (O. Bangs) [M. C. Z.]; 1 ♂, Fla., 1880 (Frederick Allen Eddy) [Univ. of Me.]; 1 ♂, Laurel Hill, N. C., L. June, 1922 (W. B. Mabee) [Am. Mus. Ac. 33827]; 1 ♂, Isld. Grove, Fla. (Siefert) [Am. Mus. No. 18900]; 1 ♂, Morehead, N. C., May 3, 1947 (Mitchell, on *Ilex*) [author's coll.]; 2 ♀ ♀, Tarpon Springs, Fla., Mar. 19, 1950 (H. K. Townes) [Townes].

According to Timberlake, this is the species recorded by Robertson (1895) erroneously as *C. thoracicus* Sm., from Inverness, Fla.

Colletes carolinus new species

Female—Length 10 mm.; face slightly longer than broad; eyes slightly divergent below; length of malar space about one-third its breadth; facial foveae quite shallow, broad, obscurely pointed on eye margin below, more rounded and slightly divergent from orbit above; antennae brownish-ferruginous beneath, second segment of flagellum subequal to pedicel in length, hardly as long as it is broad, the first segment considerably longer; lateral ocelli separated from posterior margin of vertex by about their own diameter; cheeks above very narrow; clypeus shining, punctures quite coarse and close centrally, more scattered laterally, but fine and close along the margins, a series of quite large and deep impressions just above apical margin; punctures fine and close but distinct between antennae and ocelli, minute and close on cheeks, vertex shining, the punctures exceedingly minute and obscure; labrum rather dull with about five large shallow and obscure impressions; pubescence white and quite densely plumose over most of head and thorax, a few dark hairs intermixed with the pale on vertex, mesonotum and scutellum, quite sparse on these areas except anterior half of mesonotum where there are no black hairs; pubescence entirely pale on legs; prothoracic spine not produced; metapleural protuberance not carinate; front coxae simple; mid tibiae angulate beneath toward base, hind metatarsi flat, parallel-sided, four times longer than broad; spurs yellowish-brown; tegulae shining, dark ferruginous; wings subhyaline, nervures yellowish-brown; second recurrent nervure entering third submarginal cell fully a third of the length of the cell from its apex; mesonotum and scutellum polished, punctures very fine and sparse over posterior half of mesonotum, moderately coarse and close over anterior half, closer along lateral margins, more coarse on scutellum, sparse anteriorly, becoming very close along posterior margin; pleura rather coarsely subrugose; metanotum more finely and densely rugose; posterior face of propodeum shining with a few vague irregular striations, median triangle clearly demarked by deep lateral furrows, sharply carinate above, dorsal portion short, with a transverse series of large

shining pits, lateral faces of propodeum more tessellate; abdomen shining, the punctures exceedingly minute and obscure, quite sparse on basal segment, close on the others, segments 2–4 with entire white apical fasciae, segment 1 with only a trace of such fascia at extreme sides, discs of the segments with a barely discernible covering of extremely short brown pile, a few long, erect, pale hairs toward the sides of the more apical segments, segment 6 with a few long, dark hairs.

Holotype: Female, Southport, N. C., June 24, 1928 (Mitchell, on *Hypericum*). Paratypes: 4♀♀, topotypical (Mitchell) [author's coll.]; 1♀, Albany, Ga., April 20, 1938 (P. W. Fattig) [Emory Univ.]; 10♀♀, Waycross, Ga., April 21, 1938 (F. E. Lutz); 1♀, Indian Town, Fla., Mar. 28, 1938 (F. E. Lutz); 2♀♀, Winter Park, Fla., Apr. 16, 1938 (F. E. Lutz and C. H. Curran); 1♀, Royal Palm Park, Fla., Apr. 12–18, 1923 (F.4667, Am. Mus.); 1♀, Isld. Grove, Fla., 1901 (Siefert); 1♀, Punta Gorda, Fla., Apr. 14, (Acc. 23475) [all Am. Mus.]; 2♀♀, Billy's Id., Okefenokee Swamp, Ga., June 1912 [Cornell]; 1♀, Fort Fisher, N. C., June 26, 1946 (Mitchell, on *Hypericum*).

The following note is by Timberlake:

"In Swenk's (1908) table runs out at *intermixtus*, as the venter is without scopiform hair, but it is not easily confused with that species. It is similar to my (1943) *truncatus* but larger, with the facial foveae much larger and broader, and much more sharply defined on inner side (in *truncatus* the fovea is rather narrow, with some of the strong punctures of frons invading the inner field), collar of pronotum subtruncate, but with a very sharp, slightly produced or spined anterior corner which is lacking in *truncatus;* punctures of mesoscutum a little finer, with the impunctate area of posterior middle becoming a rather sparsely punctured area, and the punctures all of one size (slightly unequal in *truncatus*); punctures of first tergite all very fine (in *truncatus* a little unequal), tergite 2 with punctures a trifle finer and sparser and without a basal band. Both of these species have the spine on front coxa too short to be placed in the *armatus* group in Swenk's key, and hence both go to *intermixtus*."

Colletes ilicis new species

Female—Length 8–11 mm.; length and breadth of face subequal; eyes convergent below; length of malar space about one-fifth its breadth; facial foveae subtriangular, rather broad, curved slightly toward lateral ocellus above and terminating about mid way between ocellus and eye; antennae dark, length and breadth of segments subequal; clypeus short, only slightly produced below suborbital line, somewhat shining, punctures close, deep and quite coarse; supraclypeal area closely and finely punctate, except for a small median spot; face above antennae densely rugoso-punctate; vertex shining, minutely punctate; cheeks minutely but more closely punctate; pubescence of head and thorax entirely pale ochraceous to whitish; lateral angles of prothorax slightly produced to form very short, sharp spines; metapleural protuberance not carinate; coxal spines lacking; legs dark, hind metatarsi about three times longer than broad; spurs pale ferruginous; tegulae reddish-piceous; wings lightly infuscated, ner-

vures and stigma piceous to ferruginous; second and third submarginal cells subequal, second receiving first recurrent nervure slightly basad of middle, third receiving the second recurrent about one-third to one-fourth from tip; mesonotum and scutellum shining, punctures deep and distinct, rather close and fine on anterior portion of mesonotum, becoming sparse posteriorly, much more coarse on scutellum, well separated but not sparse except for the impunctate anterior border; pleura dull, coarsely rugoso-punctate anteriorly and laterally, more shining, with deep, well separated punctures below; lateral faces of propodeum shining, but finely striate, dorsal face divided into a series of shining pits by short parallel striae; basal abdominal tergum shining, finely and quite closely punctate toward apical margin, punctures becoming very sparse and minute basally, the following segments more closely and minutely punctate, apical margins only very slightly depressed, very narrowly hyaline, with dense entire white fasciae, that on first tergum subinterrupted medially, apical sternum with a pair of lateral ridges; discs of terga with very short, thin, subappressed pubescence, but basal segment with some long, thin, erect, white pubescence.

Male—Length 7–8 mm.; length and breadth of face subequal; eyes convergent below; length of malar space about one-third its breadth; antennal segments fully twice as long as broad, basal segment of flagellum very short, subequal to pedicel, flagellum, except the darker basal segment, reddish-piceous; clypeus closely and rather finely punctate beneath beard; pubescence of head and thorax entirely pale ochraceous to whitish; lateral angles of prothorax slightly produced to form very short, sharp spines; metapleural protuberance not carinate; metatarsi slender, legs dark, the tarsi somewhat reddened; spurs yellowish; tegulae piceous; wings lightly infuscated, nervures and stigma piceous to ferruginous, second and third submarginal cells subequal, second receiving first recurrent nervure slightly basad of middle, third receiving the second recurrent about one-third to one-fourth from tip; mesonotum and scutellum shining, punctures deep and distinct, rather coarse and close on mesonotum laterally, well separated over most of disc, becoming sparse posteriorly, those on scutellum very much more coarse except for some small pits along the shining anterior border; pleura coarsely rugoso-punctate anteriorly and above, more shining and distinctly punctate below; lateral faces of propodeum shining, with some irregular rugosities posteriorly, dorsal area divided into a series of shining pits by the rather short, parallel striae; abdominal terga shining, minutely punctate, punctures sparse on first tergum basally, becoming close apically and on the following segments; apical margins slightly depressed toward sides, narrowly reddish-hyaline, with entire dense white fasciae, that on first segment thin or interrupted medially, discs of second and following segments with very short, subappressed, dark pubescence; wings of seventh sternum only slightly expanded apically, abruptly truncate, pubescence exceedingly short and obscure; apex of gonostyli abruptly constricted, slender, rather short pubescent; penis valves slightly broader apically than toward base, barely exceeding gonostyli in length; cuspis of volsella slender, digitus more robust, these of about equal length.

Holotype: Female, Ivanhoe, N. C., May 17, 1945 (Mitchell, on *Ilex glabra*). Allotype, topotypical. Paratypes: 2♂♂, 13♀♀, topotypical; 1♀, Ivanhoe, N. C., May 3, 1945; 4♀♀, Spout Springs, N. C., June 5, 1946 (all Mitchell, on *Ilex*) [all author's coll.]; 8♂♂, 1♀, Sand Point, Huron Co., Mich., June 22, 1922 (T. H. Hubbell) [Univ. Mich.]; 1♂, Lake Co., Mich., July 12, 1947 (R. R. Dreisbach) [Dreisbach]; 1♀, State Game Refuge, Tosco Co., Mich., July 21, 1935 (A. L. Olson—L. K. Cloyd) [Univ. Mich.]; 2♂♂, Bartow, Ga., May 31, 1931 (P. W. Fattig) [Emory Univ.]; 1♀, Ramsey, N. J., June 20, 1913 [Am. Mus.]; 2♂♂, Crescent City, Fla., Apr. 25, 1908 [Am. Mus.]; 2♂♂, McClellanville, S. C., May 10 & 15, 1944 (H. K. Townes) [Townes]; 28♀♀, Holly Shelter, N. C., May 30 & June 8, 1950 (T. B. Mitchell, on *Ilex glabra*) [Mitchell].

The following note is by Timberlake:

"This, I believe, is allied to *C. gaudialis*. The female has the hair of thorax and head much shorter and less plumose than in *gaudialis*, the facial foveae much broader and angulate within, the abdomen less hairy, with the hair bands hardly more than half as wide as they are in *gaudialis*, the second tergite without a basal band, the puncturation of abdomen a trifle finer and less dense, the pleura of thorax with punctures very close and shallow, and the last ventral segment ridged on each side. I know of no other species except *compactus* that has lateral ridges on the last ventrite."

The name *gaudialis* was proposed by Cockerell (1905) for the species erroneously identified by Viereck (1902) as *californicus* Prov.

Colletes simulans miamiensis new subspecies

Male—Length 9 mm.; length and breadth of face subequal; eyes convergent below; length of malar space about one-third its breadth; length of antennal segments about 1½ times their breadth, basal segment of flagellum much shorter than the second, flagellum brownish, but the basal segment much darker; clypeus closely and deeply punctate, upper portion hidden by the dense pubescence; face above antennae dull, rugoso-punctate, vertex shining, finely and irregularly punctured; cheeks closely and finely punctate beneath the copious pubescence; pubescence whitish on head and thorax, slightly yellowish above, mesonotum and scutellum with a few inconspicuous, fuscous hairs; lateral angles of prothorax not spined; metapleural protuberance not carinate; legs dark, metatarsi slender; spurs yellowish; tegulae yellowish-ferruginous; wings lightly infuscated, nervures and stigma piceous; third submarginal cell slightly exceeding the second, the latter receiving first recurrent slightly basad of middle, third receiving second recurrent about one-third from tip; punctures of mesonotum deep and coarse, close over most of disc, but sparse in center of posterior half; scutellum shining, coarsely and deeply punctate, anterior margin impunctate, but punctures quite close along hind margin; pleura dull, rugoso-punctate anteriorly and above, with more distinct but close and coarse punctures below; lateral and posterior faces of propodeum coarsely reticulate, dorsal face short, with closely parallel striae; base of abdomen deeply and distinctly but finely punctate, punctures well separated but not sparse; second and following terga becoming successively more

minutely and closely punctate; apical margins of terga slightly depressed, reddish-hyaline beneath the thin, white fasciae, discal pubescence on second and following terga fuscous, very short, suberect and inconspicuous; seventh sternum and genital armature essentially the same as in the other forms of *simulans*.

Holotype: Male, Miami, Fla., Jan. 21, 1924 (J. Pearson) [Am. Mus.]

The following note is by Timberlake:

"In general shows close agreement with *armatus*, but the dark hairs intermixed on mesonotum less distinct, pronotal collar truncate laterally, without a spine; punctures of tergites 1 and 2 a little finer and less dense; wings more strongly smokey (reddish) but apical margin clearer than rest of disc. Apical plates of 7th ventrite are less narrowed toward base, and the latero-basal lobe very short, barely projecting. On the whole it is probably too close to *simulans* to be regarded more than the austroriparian race of that species . . .".

Distinctions between the forms of *simulans* are given by Timberlake in his key (1943).

Colletes titusensis new species

Male—Length 8 mm.; length of face very slightly exceeding its greatest width; eyes convergent below; malar space linear, nearly obliterated; antennae dull reddish beneath, median segments short, hardly longer than broad, basal segment of flagellum much longer than the second, which is broader than long; clypeus with fine, crowded and rather deep punctures beneath the dense and rather short pubescence; face above antennae more shining, deeply, closely, distinctly and rather coarsely punctate; vertex shining, irregularly and rather closely punctate; cheeks shining between the quite close and deep punctures; pubescence of head and thorax entirely whitish, without dark admixture; lateral angles of prothorax sharply acute but not spined; metapleural protuberance not carinate; legs dark, the apical tarsal segments becoming more ferruginous, hind metatarsi rather short, length about three times the breadth; spurs yellowish-ferruginous; tegulae shining ferruginous; wings subhyaline, nervures and stigma fuscous; third submarginal cell slightly exceeding the second, the latter receiving first recurrent nervure near middle, third receiving the second about one-fourth from tip; punctures of mesonotum deep and distinct, closer over most of discs, rather coarse, becoming coarser and sparser in center posteriorly, those on scutellum coarse, deep and well separated, anterior margin shining and impunctate; pleura coarsely, deeply and closely punctate throughout; lateral faces of propodeum finely rugose, dorsal face short with shining quadrangular spaces formed by the parallel striae; abdominal terga smooth, somewhat shining, punctures minute, well separated on disc of basal segment, becoming closer and more minute and obscure on the more apical segments; apical margins of terga depressed, reddish on the more apical segments beneath the entire white fasciae, discal pubescence very short, erect, obscure and light in color; wings of seventh sternum strongly divergent, scarcely at all expanded apically, apical margin slightly rounded, each lobe considerably longer than broad; gonostyli gradually narrowed

and short pubescent at tip, much exceeded by the long, slender penis valves; cuspis of volsella slender, much elongated, greatly exceeding the short, rounded digitus.

Holotype: Male, Titusville, Fla., Apr. 25, 1923 [Am. Mus. F.4676].
This may be the male of *carolinus*.
The following note is by Timberlake:

"In my table (1943) it goes best probably to *paniscus*. . . . It differs from *paniscus* in having the clypeus finely and densely punctured all over, mesoscutum and scutellum with comparatively coarse punctures, punctures of pleura coarse and almost touching, punctures of abdomen about equally fine but closer, hair of face and thorax considerably shorter and more plumose."

Colletes thysanellae new species

Female—Length 7–9 mm.; length and breadth of face subequal; eyes convergent below; malar space extremely short, facial foveae small, rather narrow but distinct; antennae dark, length and breadth of segments subequal; clypeus produced only slightly beyond suborbital line, dull and tessellate between the rather coarse, close punctures, those on supraclypeal area fine and rather close; face above antennae dull, more tessellate than rugose, punctures indistinct; vertex shining, finely pebbled, with scattered coarser punctures, those on cheeks obscured by pubescence; pubescence whitish in general, brownish-fuscous in part on vertex and dorsum of thorax; lateral angles of prothorax produced into short, sharp spines; metapleural protuberance obscurely carinate, entirely black; anterior coxae with very short triangular spines; legs dark reddish, metatarsi about four times longer than broad; spurs yellowish-ferruginous; tegulae dark reddish; wings subhyaline, strongly violaceous, nervures and stigma ferruginous; second and third submarginal cells subequal, second receiving first recurrent slightly basad of middle, third receiving second recurrent about one-fifth from tip; dorsum of thorax shining, coarsely and deeply punctate, rather closely so on mesonotum anteriorly, becoming very sparse in center posteriorly, sparse on scutellum anteriorly, closer posteriorly, about equal in size to those of mesonotum; pleura dull, rugose anteriorly and above, becoming more striately rugose below, lateral faces of propodeum shining, microscopically reticulate, dorsal face divided into a series of rather large polished pits by the parallel striae; basal abdominal tergum shining, very minutely and sparsely punctate, those on following segments much closer, apical margins of terga only very slightly depressed toward sides, reddened, with dense entire white fasciae, discal pubescence very short, thin and entirely pale.

Male—Length 6–8 mm.; length and breadth of face subequal; eyes convergent below; length of malar space about one-fourth its breadth; antennae dark, length of segments about one and a half times the width; clypeus closely and rather coarsely punctate beneath beard; pubescence whitish in general, brownish-fuscous in part on vertex and dorsum of thorax; lateral angles of prothorax produced into very short, slender, sharp spines; metapleural protuberance obscurely carinate,

entirely black; legs dark, metatarsi slender; spurs yellowish; tegulae dark reddish; wing subhyaline and strongly violaceous, nervures and stigma ferruginous; second and third submarginal cells subequal, second receiving first recurrent slightly basad of middle, third receiving second recurrent about one-fifth from tip; dorsum of thorax shining, punctures deep and distinct, close laterally on mesonotum, rather sparse medially, becoming very sparse posteriorly, more close and coarse on scutellum, except for anterior border; pleura rugoso-punctate anteriorly and above, more shining below, obscurely, striately punctate; lateral faces of propodeum somewhat shining, obscurely and irregularly sculptured, dorsal face divided into several shining quadrate pits by the parallel striae; basal abdominal tergum shining, sparsely and very finely punctate, those on following segments closer, becoming minute and obscure apically, apical margins of terga depressed, reddish-hyaline, with dense entire white fasciae, discal pubescence of second and following segments dark, very fine, obscure and subappressed; wings of seventh sternum quite broadly expanded and truncate apically, short pubescent near base, but this becoming very thin and obscure toward center apically; gonostyli very strongly and abruptly constricted apically, this finger-like projection with pubescence of moderate length; penis valves quite broad, slightly expanded toward base, considerably exceeding gonostyli; cuspis of volsella slender, the digitus robust and slightly longer.

Holotype: Female, Pensacola, Fla., Oct. 11–14, 1914 [Am. Mus.]. Allotype, topotypical. Paratypes: 19♂♂, topotypical; 1♀, Pablo Beach, Fla., Nov. 4, 1911; 2♂♂, Miami, Fla., Oct. 21 & 23, 1934 (J. Pearson); 1♀, Coral Gables, Fla., Nov. 4, 1934 (H. Pearson) [all Am. Mus.]; 9♀♀, Inverness, Fla., 1918 (Charles Robertson) [Ill. Nat. Hist. Surv.]; 1♂, St. Simon's Isld., Ga., Oct. 22, 1910 [Cornell].

The series of females from Inverness, Fla., in the Robertson collection, was found under the name *thysanellae*, but the description was never published.

Colletes bradleyi new species

Female—Length 9 mm.; length and breadth of face subequal; eyes converging below; malar space very short, linear; facial foveae well developed, subtriangular, rounded above, broad medially, more pointed below; antennae ferruginous beneath, length and breadth of mid antennal segments subequal; clypeus produced but very slightly below suborbital line, shining, deeply and rather coarsely and closely punctate; supraclypeal area shining and impunctate medially, becoming finely and closely punctate laterally; face above antennae rather dull, punctures irregular, rather shallow, interspaces tessellate; vertex shining, punctures scattered, very minute; cheeks closely and very finely punctate; pubescence of head and thorax entirely pale, whitish below, more creamy above; lateral angles of prothorax not spined; metapleural protuberance carinate, margin of the carina narrowly testaceous; anterior coxal spines lacking; tarsi slightly reddened but not contrasting with tibiae or femora, length of hind metatarsi about three times

their breadth; tegulae ferruginous; wings subhyaline, violaceous, nervures and stigma ferruginous; third submarginal cell slightly exceeding the second, the latter receiving first recurrent nervure slightly basad of middle, the third receiving the second recurrent about one-fourth from tip; thorax shining, punctures of mesonotum fine and quite sparse, even anteriorly, becoming more sparse and slightly more coarse and deep posteriorly; punctures of scutellum much more coarse, close posteriorly, becoming fine and sparse anteriorly; punctures of pleura deep, rather coarse, well separated but not sparse; lateral faces of propodeum somewhat shining, obscurely sculptured posteriorly, dorsal face divided into eight or nine shining pits by the parallel striae; basal abdominal tergum shining, finely but distinctly punctate, punctures well separated but not sparse, following segments becoming successively more finely and closely punctate; apical margins not appreciably depressed but reddish-hyaline beneath the entire, rather narrow but dense white fasciae, discal pubescence erect, very short and thin, faintly brownish.

Holotype: Female, Chatsworth, Burlington Co., N. J., June 15, 1923 (J. C. Bradley) [Cornell Univ.]. Paratype: 1 ♀, Chesilhurst, N. J., June 3, 1939 (H. K. Townes) [author's coll.]

The following note is by Timberlake:

"This must be considered to belong to the *americanus* group on account of the short malar space, ciliate inner spur of hind tibia, and the testaceous margined overhang on upper part of metapleura, but differs in lacking the hairy spine on front coxa. The sparse hair of head and thorax and the very narrow abdominal bands are distinctive also in comparison with other species of the *americanus* group."

Hylaeus graenicheri new species

Female—Length: 4 mm.; thorax mostly black, head above and laterally black, abdomen chiefly red, but apical terga blackish; antennae, including scape, ferruginous, paler beneath; clypeus yellowish in center, more ferruginous around the margin; face marks yellow, narrow, elongate, entirely filling area between clypeus and eyes, extending along inner orbits nearly to top of eyes; mandibles ferruginous, labrum somewhat darker; collar and tubercles yellow; tegulae pale ferruginous, yellow anteriorly; legs reddened, front tibiae yellow anteriorly, basal third of hind tibiae yellowish; spurs yellowish; face narrow and elongate; eyes much converging below; cheeks slightly narrower than eyes; foveae short, extending from tip of yellow face mark to top of eye, separated from eye by a space of about equal width, little if any divergent from eye above; segments of flagellum slightly broader than long, the more apical ones about as long as broad; front coxae simple; dorsal area of propodeum slightly longer than metanotum, finely tessellate but otherwise smooth, posterior face truncate, subcarinate laterally, finely and obscurely reticulate, lateral surfaces tessellate or finely subrugose; metanotum somewhat shining; clypeus obscurely tessellate, impunctate; face above antennae very finely rugoso-punctate; mesonotum finely and quite closely

punctate, dull; scutellum more shining, punctures less close and more coarse; pleura tessellate, punctures rather fine and sparse; abdomen impunctate.

Type—Female, Miami, Fla., Dec. 22, 1916 (S. Graenicher) [author's coll.].

As this unique specimen is a female, there is no basis for judgment as to its systematic position, and therefore it is not assigned to any subgenus.

Hylaeus (Prosopis) certus new species

Female—Length: 6 mm.; black; antennae black above, piceous below; face marks yellow, triangular, entirely filling area between clypeus, eyes and antennae, terminating obliquely on eye margin at level of antennae; tubercles and two conspicuous maculae on collar yellow; tegulae piceous, not maculated; wings subhyaline, nervures and stigma piceous; front and mid tibiae with basal yellow spots slightly more than a third of their length; slightly more than basal half of hind tibiae yellow; spurs yellowish; legs otherwise black; face somewhat narrowed below; eyes slightly convergent; cheeks subequal to eyes in width in lateral view; foveae linear, deep and distinct, separated from eye by a space of about equal width, markedly divergent from eye above; inner orbits slightly elevated above level of eyes; second segment of flagellum slightly broader than long, these dimensions subequal in first and third segments, following segments slightly longer; front coxae simple; dorsal area of propodeum about as long as metanotum, coarsely reticulate, as also the posterior surface which is bounded by a low carina and with a deep median groove, surface somewhat obscured by greyish tomentum, lateral surfaces finely rugose; metanotum densely and finely rugose; face below antennae tessellate, sparsely and very shallowly and obscurely punctate; surface above antennae closely, deeply and rather coarsely punctate, subrugose; mesonotum quite coarsely and deeply punctate, close but not crowded, tessellate between punctures; punctures of scutellum slightly more sparse, deep and separate; pleura dull, densely tessellate, with coarse, shallow, well separated punctures; basal segment of abdomen shining, nearly impunctate, second and following segments less shining, with rather close and very minute punctures.

Male—Black; antennae blackish above, brownish beneath; scape, mandibles and labrum black; tegulae piceous, not maculated; wings subhyaline, nervures and stigma brownish; maculations yellow, as follows: entire face below antennae, supraclypeal mark subquadrate, somewhat rounded above between antennae, face marks contiguous with antennal foramen on outer side, obliquely truncate just above antennae, tubercles and two small spots on collar, all the tibiae, tarsi and spurs, but front and mid tibiae with large ferruginous blotches on under side, partially encircling mid tibiae; face narrowed below; eyes convergent; cheeks slightly narrower than eyes; scape somewhat robust, diameter at apex considerably exceeding diameter of flagellum, first and second segments of flagellum about as long as broad, following segments somewhat longer; front coxae simple; dorsal area of propodeum slightly longer than metanotum, very coarsely reticulate, as also posterior surface which is delimited by a low, barely evident carina, this interrupted medially above, lateral surfaces finely irregularly rugose; punctures

of mesonotum deep and quite coarse, crowded anteriorly, close over remainder of disc, well separated but not sparse on scutellum; quite coarse and well separated on pleura; basal segment of abdomen shining, impunctate, second and following segments closely and very minutely punctate; wings of seventh sternum crescentic, the outer concave margin fringed with fine setae; distal pedicel of eighth sternum rather narrow, truncate apically; penis valves of genital armature not attaining tips of gonostyli, the latter rather robust, narrowly rounded apically.

Type—Male, W. Nyack, N. Y., June 5, 1938 (A. L. Melander) [M.C.Z.]. Allotype, Holliston, Mass., July, 1941 (Banks. on cultivated parsnip) [M.C.Z.]. Paratypes: 1 ♀ Huguenot, N. Y., Aug. 10, 1904 (J. L. Zabriskie) [A.M.N.H.]; 1 ♂, Tappan, N. Y., June 10, 1920 [A.M.N.H.]; 1 ♂, W. Hernden, Me. (Frederick Allen Eddy); 1 ♂, Princeton, Me., July 2; 1 ♂, Calais, Me., July 10 [all M.C.Z.]; 1 ♂, Needham, Mass., June 17, 1925 (Mitchell, on *Apocynum*) [author's coll.].

Hylaeus (Prosopis) ornatus new species

Female—Length: 6–8 mm.; black, with basal segment of abdomen bright ferruginous; antennae blackish above, reddish beneath; face marks yellow, triangular, entirely filling area between clypeus, eyes and antennae, terminated acutely above antennae, tip slightly divergent from eye margin; collar with large yellow maculae, and tubercles yellow; tegulae dark brown, sometimes maculated; wings hyaline at base, becoming infuscated apically, nervures and stigma fuscous; femora blackish or fuscous; front tibiae yellowish at base, otherwise ferruginous, basal third of mid tibiae and basal half of hind tibiae yellowish, apically dull ferruginous; all tarsi ferruginous; face narrowed below; eyes convergent; cheeks about as broad as eyes in lateral view; foveae deep, distinct, linear, separated from eye by about an equal space, slightly divergent from eye above; second and third segments of flagellum slightly broader than long, others about as long as broad; front coxae simple; dorsal area of propodeum about as long as metanotum, very coarsely reticulate, posterior surface coarsely reticulate, bordered by a low carina, but divided by a deep median longitudinal groove which is broadened above, lateral faces finely rugose; metanotum densely rugose; face below antennae tessellate, with sparse, shallow, obscure punctures, deeply and closely punctate above antennae, subrugose; punctures of mesonotum and scutellum deep, distinct, rather close and quite coarse; pleural punctures very coarse but rather shallow, close but not contiguous, interspaces tessellate; basal segment of abdomen shining, impunctate, following segments also to some degree shining, but with very minute and rather close punctures.

Male—Black, with basal segment of abdomen bright ferruginous; antennae fuscous above, ferruginous beneath, scape black; mandibles yellowish in part; labrum black; tegulae entirely fuscous; wings hyaline at base, becoming infuscated apically, nervures and stigma fuscous; maculations yellow, as follows; entire face below antennae, supraclypeal mark triangular above, face marks

extending along eye margin above antennae and truncate or rounded apically, conspicuous transverse maculae on collar, tubercles, all tibiae and tarsi except that front and mid tibiae have a reddish blotch beneath, and all spurs yellow; face narrowed below; eyes convergent; cheeks narrower than eyes; scape rather robust, diameter at apex about one and a half times that of flagellum, basal segment of flagellum slightly shorter than broad, these dimensions subequal on second segment, third and following segments considerably longer; front coxae simple; dorsal area of propodeum sloping, about as long as metanotum, very coarsely reticulate, substriate, propodeum otherwise quite coarsely reticulate, posterior face bordered by a quite distinct carina, this interrupted above by a central pit or excavation; metanotum coarsely rugose; mesonotum coarsely and very closely punctate, punctures well separated on scutellum, pleura very coarsely rugose; basal segment of abdomen shining, practically impunctate, second and following segments with minute and rather close punctures; wings of seventh sternum subtriangular, outer slightly concave margin fringed with fine setae, apical angle attenuated, slender and elongate; distal pedicel of eighth sternum quite narrow, the truncate tip slightly incurved; penis valves of genital armature quite slender, much shorter than gonostyli, these rather robust, narrowly rounded apically.

Type—Male, Swan Quarter, North Carolina, June 19, 1945 (Mitchell, on *Hypericum*). Allotype, Goldsboro, North Carolina, July 28, 1921 (Mitchell) [both author's coll.]. Paratypes: 1♂, topotypical; 2♀♀, Wilmington, N. C., June 30, 1923 (on *Cephalanthus*), and June 27, 1946; 1♀, Nags Head, June 20, 1946; 8♂♂, Core Point, Beaufort, Co., N. C., July 17, 1948; 3♂♂, Core Point, N. C., Sept. 4 & 5, 1948 (all Mitchell) [all author's coll.]; 1♂, Raleigh, N. C., mid July, 1912 (C. L. Metcalf) [N. C. Dept. Agr.]; 1♂, 3♀♀, Kill Devil Hills, N. C. June 28, 1950 (K. V. Krombein, on Virginia creeper); 1♂, Kill Devil Hills, June 30, 1950 (Krombein) [U.S.N.M.].

This species is very close to *confluens*, Smith (1853), but seems to show constant differences, even though these differences may seem to be minor. The red base of the abdomen will at once separate *ornatus* from *confluens*, and the tegulae are nearly always entirely dark, being maculated in *confluens*. Furthermore, differences in the sculpture of the propodeum, as described, seem to be constant.

Because of the red base of the abdomen, this species has been confused with *nelumbonis*, Robertson (1890). The male differs from that of *nelumbonis* by the maculated collar, this being entirely black in *nelumbonis*, and by the entirely yellow mid and hind tibiae which in *nelumbonis* are dark apically, yellow only over basal half at most. The pleura in *nelumbonis* are somewhat more densely rugose than in *ornatus*. The genitalia and ventral plates are very similar in the two species. The females differ most conspicuously by the large yellow maculae of the collar and the distinctly separated pleural punctures of *ornatus*, the pleura being densely rugose and the collar entirely black in *nelumbonis*.

Hylaeus (Metziella) hydrangeae new species

Female—Length: 4.5 mm.; black; antennae piceous above, ferruginous beneath; face marks yellow, subtriangular, nearly filling space between clypeus and eye, but short, terminated by a recurved line extending from upper corner of clypeus to eye at level of antennae; tubercles yellow; collar yellow laterally, these maculae separated by about an equal area of black; tegulae brownish, with a small yellow anterior spot; wings lightly infuscated, nervures and stigma brownish; front and mid tibiae with a basal yellow spot, basal third of hind tibiae yellow, legs otherwise dark; spurs yellowish; face broad below; eyes slightly convergent below; facial foveae quite fine, linear, lower end considerably above level of antennae, upper end strongly divergent from eye, ending at about mid point between eye and lateral ocellus; basal segment of flagellum slightly shorter than pedicel, about as long as broad, second segment much shorter, broader than long, following segments becoming successively longer; cheeks and eyes subequal in width in lateral view; front coxae triangular on outer side, the apex somewhat rounded, not at all spinose; punctures of clypeus and supraclypeal area very sparse and obscure, nearly impunctate, close and fine but shallow and obscure above antennae, on cheeks and on vertex, fine and rather close on mesonotum and scutellum, somewhat more sparse on pleura; dorsal area of propodeum extensive, fully twice as long as the metanotum, with coarse, more or less parallel striae; abdomen not visibly punctate, basal segment shining.

Type—Female, Cruso, North Carolina, June 25, 1934 (Mitchell, on *Hydrangea*).

This is very similar to *sparsa*, Cresson (1868), but is much smaller, and the different form of the propodeum would indicate that the two are distinct.

Hylaeus (Paraprosopis) packardi new species

Male—Length: 4 mm.; black; antennae brownish-ferruginous, paler beneath, scape piceous; tegulae piceous; wings subhyaline, nervures brownish; mandibles and labrum black; maculations cream-colored as follows: clypeus, supraclypeal area, lateral portions of face extending along inner orbits and ending acutely on eye margin above antennae, small spot on tubercles, entire front tibiae except a slight amount of ferruginous posteriorly, basal fourth and apical rim on mid and hind tibiae, all tarsi except the reddened more apical segments; collar and tegulae entirely dark; eyes convergent below; cheeks slightly more than half the width of eyes in lateral view; thoracic punctures deep and distinct, close but not contiguous on mesonotum and scutellum, slightly more coarse on pleura, interspaces dull and tessellate; abdomen shining at base, punctures minute, well separated, becoming closer but very obscure on the following segments which are less shining.

Type—Male, Troy, N. Y., Sept. 2, 1934 (H. K. Townes) [author's coll.]. Paratype: 1♂, Brunswick, Me., (A. S. Packard) [M.C.Z.].

This species is very closely related to *eulophi*, Robertson (1905), and *wootoni* Cockerell (1895), and may prove to be only a variant of one of those species.

It is easily separated from *eulophi* by the entirely black unmaculated collar and tegulae. In *wootoni* the tegulae are unmaculated, but the collar evidently is maculated. The facial marks more nearly resemble those described for *wootoni*. The genital structures are very similar to those of *eulophi*.

Hylaeus (Prosopis) volusiensis new species

Female—Length: 8 mm.; black, including antennae, labrum, mandibles, entire face above clypeus and face marks; triangular lateral face marks, tubercles and two maculae on collar ivory; clypeus ferruginous; tegulae with ferruginous maculae; wings subhyaline basally, becoming slightly infuscated apically, nervures and stigma fuscous to blackish; first and second segments of abdomen brownish-ferruginous, the second with a narrow, apical blackish rim, the third brownish-ferruginous at extreme base, abdomen otherwise blackish; legs in large part brownish-ferruginous, the front and middle femora somewhat blackened; face somewhat narrowed below, the eyes convergent; cheeks slightly narrower than eyes; facial foveae extremely narrow but quite distinct, closely paralleling upper margin of eyes, slightly diverging from eye margin at upper end; basal segment of flagellum subequal to pedicel, the second segment slightly shorter, following segments slightly increasing in length; punctures of face shallow, well separated on areas below antennae, more close and fine but obscure above, very fine, shallow and obscure on vertex and cheeks; punctures of mesonotum slightly more coarse, deep and distinct, not crowded, those on scutellum slightly more sparse, the surface rather smooth, pleural punctures rather indistinct, shallow and irregular; abdominal terga practically impunctate.

Male—Black, including labrum, mandibles, and antennae above, flagellum more piceous beneath; basal segment of abdomen and all tibiae and tarsi brownish-ferruginous, apical portions of femora of the same color; tegulae brownish, the wings subhyaline basally, becoming somewhat infuscated apically, nervures and stigma piceous; clypeus, supraclypeal area, lateral face marks, tubercles and two small spots on collar ivory, the lateral face marks extending slightly above antennae, rounded; scape not maculated; face narrowed below, the eyes convergent; cheeks narrower than eyes, basal segment of flagellum subequal to pedicel in length, the second and following segments becoming slightly longer; punctures of maculated areas of face fine, but rather deep and distinct, well separated but not sparse, those above antennae very close and fine, but rather deep and distinct, slightly separated on vertex, becoming somewhat more sparse on cheeks below; punctures of mesonotum rather close, deep and distinct, those on scutellum somewhat more sparse and coarse, the surface moderately shining; pleural punctures slightly more coarse and more distinctly separated than those on mesonotum; metanotum finely rugose; dorsal area of propodeum rather coarsely rugoso-striate, lateral areas smoother, only finely roughened, punctures obscure, posterior face with a rather deep median sulcus; abdominal segments quite smooth and shining, punctures exceedingly minute, barely visible.

Type—Female, Volusia County, Florida, March 28, 1930 (J. P. Turner—

Florida Fruit Fly Trap Survey) [U.S.N.M.]. Allotype—Male, topotypical, March 7, 1930 (O. B. Webb) [U.S.N.M.].

In structural characters, *volusiensis* is very close to *certus*, the male genital structures being very similar. The color differences, however, are marked, and the type localities are widely separated, so it seems best at this time to consider the two as being distinct species, recognizing the possibility that additional material and future studies may show that they are only subspecifically different.

Andrena macra new species

Female—Length 11 mm.; length and breadth of face subequal; clypeus only slightly convex, projecting about one-half below suborbital line, dull, the punctures fine and quite close, without a distinct median impunctate line; facial foveae broad above, occupying most of area between eyes and lateral ocelli, covered with pale ochraceous tomentum; space between margin of vertex and lateral ocelli slightly shorter than distance between them; cheeks broader than eyes, rounded posteriorly, rather dull, the punctures exceedingly minute and obscure; malar space exceedingly short; basal segment of flagellum subequal to second and third segments combined; process of labrum rather small, width about twice the length, subtriangular, narrowly truncate apically; pubescence of head, of thorax laterally, and of legs pale ochraceous, that on dorsum of thorax pale fulvous, rather short, and dense; mesonotum very closely and finely punctate, punctures becoming somewhat more sparse in center posteriorly, those on scutellum slightly more coarse, deep and distinct, well separated on each side of middle, but otherwise close, surface shining; pleura dull, densely tessellate, very obscurely punctate; enclosure of propodeum dull, densely tessellate, obscurely subrugose toward basal margin; propodeal corbicula well developed, rather short, anterior fringe rather poorly developed, pale ochraceous; trochanteral floccus long, well developed, whitish; tibial scopa dense, pale fulvous to ochraceous, hairs rather elongate, simple; pubescence of basal leg joints whitish; that on tibiae and tarsi becoming ochraceous, metatarsi very slightly narrower than their respective tibiae; second submarginal cell slightly shorter than third, receiving first recurrent slightly beyond middle; abdominal terga shining, apical depressed areas rather obscure, occupying medially about one-third the length of the discs, becoming reddish-hyaline toward rim, the discs uniformly, deeply, finely and very closely punctate, discal pubescence extremely short, suberect, entirely whitish, white fasciae evident toward sides, widely interrupted medially on second and third segments, fifth tergum with a dense apical ochraceous fimbria.

Male—Length 9 mm.; length and breadth of face subequal; clypeus quite flat, projecting but very little below suborbital line, dull, very finely and quite closely punctate beneath the dense pubescence; space between margin of vertex and lateral ocelli somewhat shorter than distance between them, only slightly exceeding their diameter; cheeks much broader than eyes, obscurely angulate opposite middle of eye, the angle rounded, surface rather dull, minutely and

obscurely punctate; malar space extremely short; basal segment of flagellum longer than second segment, slightly shorter than second and third combined; process of labrum subquadrate, slightly narrowed apically, the truncate apex slightly incised; mandibles quite slender, slightly flexed, with a distinct, rather small, inner subapical tooth, but without a basal inferior projection, overlapping about one-third; pubescence of head, thorax, and legs white; mesonotum quite finely, closely and deeply punctate anteriorly, somewhat more sparsely so in middle posteriorly where the surface is more shining; scutellum somewhat shining, the punctures very fine, scattered, and irregular, rather sparse in center; pleura very dull, tessellate, with numerous rather coarse and close punctures on upper half; enclosure of propodeum dull, tessellate, obscurely subrugose toward basal margin; all metatarsi slender and elongate, considerably narrower than their respective tibiae; second submarginal cell slightly shorter than third, receiving first recurrent slightly beyond middle; abdominal terga shining, apical margins rather narrowly and obscurely depressed, becoming reddish-hyaline toward rims, punctures of discs uniformly fine, deep and distinct, rather close except near middle, discal pubescence very short, suberect, whitish, with whitish apical fasciae evident only toward sides on second and third segments; apical portion of eighth sternum rather slender, sides nearly parallel, with a very low median ventral ridge beyond which it is quite densely pubescent to the rather narrowly truncate tip; aedagus slender, only slightly expanded basally, not excavated, parameres quite slender and elongate, very slightly dilated apically, parapenial lobes not at all produced.

Type—Female, Raleigh, N. C., May 16, 1925 (Mitchell, on *Rubus*). Allotype, Raleigh, N. C., Apr. 20, 1946 (Mitchell, at nest site). Paratypes: (all Raleigh, N. C.) 1♂, Apr. 13, 1935 (H. F. Schoof); 4♀♀, Apr. 28, 1940; 1♀, May 8, 1940; 1♂, May 9, 1940; 1♀, May 11, 1940; 1♂, Apr. 14, 1942; 1♀, 4♂♂, Apr. 3, 1945 (at nest site); 3♀♀, 5♂♂, Apr. 10, 1945 (at nest site); 9♂♂, Apr. 20, 1946 (at nest site); 1♀, Apr. 21, 1946 (on *Rubus*); 1♀, May 19, 1946; 1♀, Apr. 27, 1947; 1♀, May 19, 1950 (at nest site); [all author's coll.].

The name *macra* was applied by Viereck to a specimen of this species (not now at hand) in the collection of the Philadelphia Academy of Science, but the description was never published. This species seems to come nearest to those included by Lanham (1949) in the subgenus *Gymnandrena*, although the condition of the trochanteral floccus is not quite characteristic of that group, being more nearly perfect as in *Cryptandrena*. The abdominal terga, also, are considerably more deeply, coarsely and closely punctate than in any of the other species of either of these groups that have been examined.

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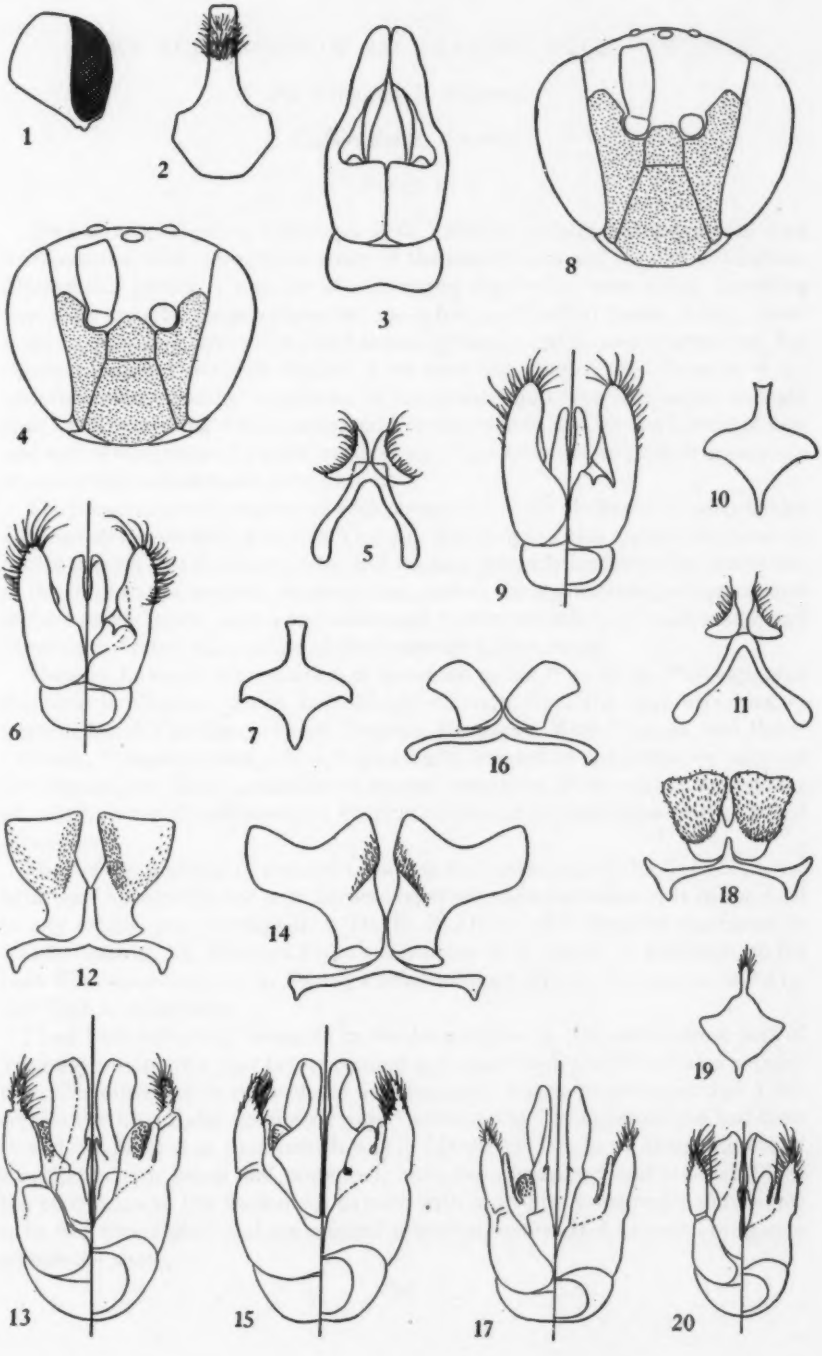
EXPLANATION OF PLATE

PLATE 16

FIG. 1. Andrena macra, male, lateral view of head.
FIG. 2. Andrena macra, male, eighth sternum.
FIG. 3. Andrena macra, male, genital armature.
FIG. 4. Hylaeus certus, male, anterior view of head.
FIG. 5. Hylaeus certus, male, seventh sternum.
FIG. 6. Hylaeus certus, male, genital armature.
FIG. 7. Hylaeus certus, male, eighth sternum.
FIG. 8. Hylaeus ornatus, male, anterior view of head.
FIG. 9. Hylaeus ornatus, male, genital armature.
FIG. 10. Hylaeus ornatus, male, eighth sternum.
FIG. 11. Hylaeus ornatus, male, seventh sternum.
FIG. 12. Colletes ilicis, male, seventh sternum.
FIG. 13. Colletes ilicis, male, genital armature.
FIG. 14. Colletes brimleyi, male, seventh sternum.
FIG. 15. Colletes brimleyi, male, genital armature.
FIG. 16. Colletes titusensis, male, seventh sternum.
FIG. 17. Colletes titusensis, male, genital armature.
FIG. 18. Colletes thysanellae, male, seventh sternum.
FIG. 19. Colletes thysanellae, male, eighth sternum.
FIG. 20. Colletes thysanellae, male, genital armature.

PLATE 16



A NEW SUBSPECIES OF SALAMANDER FROM VIRGINIA

By Richard L. Hoffman

Clifton Forge, Virginia

Plate 17

For a number of years, starting in 1943, I carried on fairly extensive field work in connection with a projected study of the amphibians and reptiles of Virginia. During this period, a number of interesting discoveries were made, involving new state records, range extensions, and a few undescribed forms. Lately, however, an increasing interest in other animal groups, more in need of attention, has caused an almost complete neglect of the state herpesian survey. Because of the uncertain and unsettled conditions of the present and future, it seems unlikely that I will ever bring it to completion. For this reason, and for the benefit of new and active workers on Virginia herpetology, I have decided to publish separately a part of the accumulated information.

The present paper is concerned with occurrence of the plethodontid salamander *Desmognathus monticola* Dunn in Virginia. The range of this species has been recently treated by Grobman (1945) and his map properly indicates the restriction of the form to the western, mountainous, part of the state. Since the appearance of Grobman's paper, only a few additional county records have been made, and these do not affect the outline of the presently known range.

There is, however, a population of *monticola* in the Blue Ridge Physiographic Province in Virginia, which is strikingly different from the form occurring in western North Carolina, western Virginia, Kentucky, West Virginia, and Pennsylvania. This population, which is genetically isolated by the extensive valley of the Shenandoah River, maintains a general constancy of characters throughout its range, except in southwestern Virginia where the physiographic barrier is not pronounced.

Most of the material of the new form is in the collections of the U. S. National Museum. Apparently the only herpetologist who has encountered it in the field to any extent, prior to myself, is Dr. E. R. Dunn, who obtained specimens in Nelson County, Va. Much of Dunn's discussion of *D. phoca* (= *monticola*) in his book *The Salamanders of the Family Plethodontidae* (1926, p. 73 *et seq.*) is based on the Virginia subspecies.

I had been collecting *monticola* in the Alleghanies (in the westernmost part of Virginia) for about a year before coming in contact with the Blue Ridge population. The difference in color of the living animals was so pronounced that I felt certain the Blue Ridge specimens were "nameworthy" even before one had been picked up. Since that time (which was in March 1945) a fairly large number of specimens, both living and preserved, have been examined and studied. Since the correlation of the distinctive pattern with a definite geomorphic unit seems to be well established, and my original suspicion confirmed, I herewith propose a subspecific name.

Desmognathus monticola jeffersoni, new subspecies

Virginia Synonymy

Desmognathus monticola Dunn, 1917, Proc. U. S. Nat. Mus., vol. 53, p. 404 (in part); 1918, *Copeia*, no. 53, p. 19 (in part); 1920, Proc. Biol. Soc. Washington, vol. 33, p. 132 (in part).

Desmognathus phoca Dunn (nec Matthes), 1926, *Plethodontidae*, p. 73 (in part); 1936, List of Virginia amphibians and reptiles, mimeographed, p. 1 (Haverford, Pa.); Brady, 1924, *Copeia*, no. 126, p. 29.

Type specimen.—U. S. National Museum no. 126891, adult female, collected in Saddle Hollow on Jarman's Mountain, 2 miles west of Crozet, Albemarle County, Virginia (elevation 1600 feet), on March 11, 1945, by R. L. Hoffman (field no. 23).

Paratype specimens.—U. S. N. M. nos. 126892–96, March 11, 1945; nos. 123759–61, March 27, 1946; R.L.H. nos. 370–75, September 27, 1945, the preceding all topoparatypes. R.L.H. nos. 54–55, from Charlottesville, Albemarle Co., Virginia, March 25, 1945.

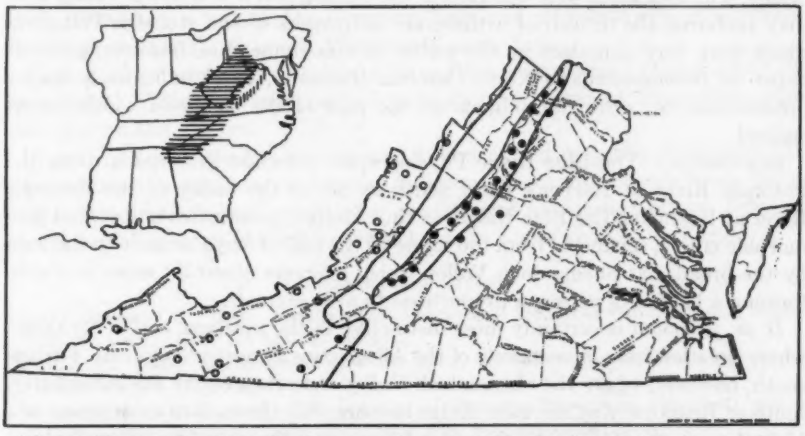
Diagnosis.—Similar to *D. m. monticola* Dunn in structural characters, differing primarily in color pattern. The black pigment of the dorsal markings is much reduced to a very sparse and irregular scattering of black spots, sometimes absent in adults. This pattern may be summarized as follows: venter grayish-white, unmottled; dorsum pale grayish-brown, with a variable number of rounded black spots (about size of eye) scattered at random on back and sides. Head slightly darker than back, with several black flecks behind the eyes. The usual diagonal yellow mark from eye to angle of jaw, lightly edged in black. No sharp demarcation between color of venter and that of lower sides, the latter faintly mottled. Tail flecked with dark pigment laterally, the keel orange-buff. Limbs uniformly light brown except for darker suffusion at joints. The pattern of the type is figured (Pl. 17, fig. 1); compare it with that of a normal specimen from western Virginia (fig. 2) and with Bishop's figure (1943, *Handbook of Salamanders*, fig. 56, p. 209) of a Kentucky specimen.

Variation.—There is a certain amount of variation in this subspecies with respect to both structural features and color pattern. In the material studied closely (about 40 specimens) vomerine teeth (the sum of both sides) range from 0 to 13 in males, and from 9 to 17 in females, averaging 4.9 and 12.7, respectively. Costal grooves (maximum count) vary between 13 and 16, the numbers 13 and 14 occurring most frequently (85%).

It is difficult to define variations of pattern except pictorially. The holotype, of which a photograph is given, is representative of the most common color pattern, from which there are a certain number of deviations in both directions. Thus, in a few old specimens the black spots are completely absent and the animal is uniformly fawn-colored. Ontogenetic variation results in the young salamanders having considerably more pigment in the pattern; however, juveniles of *jeffersoni* are considerably lighter in color than specimens of comparable size from else-

where in the range of *monticola*. Such differences, being qualitative, are best appreciated through actual comparison of living specimens, which I have been able to make from time to time. There seems to be no sexual variation in the relative development of the color pattern.

There is no evident geographic variation of pattern within the range of *D. m. jeffersoni*. In the southern part, however, as in Floyd Co., specimens appear to be intermediate between typical *jeffersoni* and *D. m. monticola*. Probably a long time has passed since any continuity of range has been maintained along the Blue Ridge (due to the broad low watergap of the Roanoke River), so that the matter of actual intergradation seems improbable.



MAP 1. Virginia localities for *Desmognathus monticola* showing distribution of *D. m. monticola* (hollow circles) and *D. m. jeffersoni* (solid circles). The two half-shaded circles represent intermediate samples. The solid line around the *jeffersoni* spots indicates the extent of the Blue Ridge Province in Virginia north of the Roanoke River. The solid line bounding the *monticola* localities follows the western edge of the Shenandoah and Roanoke River valleys, and the eastern escarpment of the Blue Ridge south of Roanoke.

The inset outline map of eastern United States shows the over-all relationship of the range of *jeffersoni* (vertical shading) to that of *monticola* (horizontal shading). The latter is probably composed of two or more subspecies not yet recognized.

Ecological notes.—*D. m. jeffersoni* is a salamander of steep cold mountain streams. Typically it is found in company with *Gyrinophilus porphyriticus* and where these two salamanders are common, as at the type locality, other species are notably scarce. In Saddle Hollow only a few specimens of *D. f. fuscus* have been found (these well away from the stream) and *Eurycea b. bislineata*, which occurs in the same stream, is found only at lower elevations where the larger salamanders do not descend.

Dunn (1926, Plethodontidae, p. 74) records that in Nelson Co., Va., this form was found in deep shady ravines cutting back from the James River, where conditions were similar to those found at higher elevations. In Albemarle County,

and doubtless elsewhere as well, *jeffersoni* occurs away from the Blue Ridge in small outlying parallel ridges which are about fifteen miles east of the main body of the mountains. Ecologically they are much like the larger mountains in that they provide steep, well-aerated creeks. The intervening area, which is flat with warm sluggish streams, is completely devoid of *jeffersoni*.

Invertebrates found associated with *jeffersoni* are the usual inhabitants of lotic situations, and include the nymphs of stoneflies and mayflies, and larvae of beetles and caddisflies. No survey of stomach contents has yet been made, but it is of interest to note that two topotypes collected in March 1948 contained only millipeds, *Scytonotus virginicus* (Loomis), and isopods, *Ligidium longicaudatum* Stoller. The specimens were collected in the stream, and it is thus surprising that they preferred the terrestrial arthropods to nymphs of the stonefly *Peltoperla* which were very abundant in the water. In this connection, however, a recent paper on *Desmognathus* in North Carolina (Hairston, 1949) indicates a similar predilection for terrestrial animals on the part of the *monticola* which he examined.

Distribution.—The Blue Ridge Physiographic Province in Virginia, from the Potomac River at Harper's Ferry south as far as the valley of the Roanoke River at Roanoke. The Blue Ridge, as thus limited, constitutes an isolated geomorphic entity, separated from the ridges of the folded Appalachians to the west by the broad, flat Shenandoah Valley which averages about 30 miles in width, forming a barrier of no mean proportions to lotic animals.

D. m. jeffersoni is certainly most distinctive in the northern end of its range, where isolation from *m. monticola* of the Alleghanies is most pronounced. Further south, however, where the Shenandoah Valley loses its identity (as immediately south of Roanoke) and the Blue Ridge becomes but the eastern escarpment of a high plateau, specimens tend to appear intermediate between the two subspecies.

It is interesting to note that although the Potomac River seems to be the northern barrier for *jeffersoni*, it does not affect the northward extension of *monticola* in the Alleghanies.

Specimens examined.—110, all from Virginia, as follows:

Albemarle County: Saddle Hollow, west of Crozet, USNM 123759–61, 126891–96, RLH 370–75; Sugar Hollow, 6 miles west of Whitehall, RLH 1176–79; Charlottesville, RLH 54–55, 494; Covesville, RLH 78–91.

Augusta County: Calf Mountain, 4 miles east of Waynesboro, USNM 124340.

Bedford County: Bedford, USNM 99064–82; Big Island, USNM 118324–25; Wheat's Valley, USNM 99083; Blue Ridge Parkway, 4 miles south of Apple Orchard Mountain, RLH 1180.

Clarke County: "Blue Ridge," USNM 94999–95003; Mount Weather, USNM 99009–912.

Fauquier County: Delaplane, USNM 39024–25.

Greene County: east side of Swift Run Gap, RLH 1075.

Madison County: Robertson River, USNM 118323.

Page County: Stony Man Mountain, USNM 29252; Thornton Gap, USNM 84371–89; west side of Thornton Gap, USNM 59899–915.

Rappahannock County: 3 miles east of Thornton Gap USNM 103192–94.

Rockbridge County: Blue Ridge Parkway, 10 miles north of intersection with U. S. Rt. 60, RLH 1193–96.

Warren County: Shenandoah National Park, east of Front Royal, J. A. Fowler collection, no. 71.
"Skyline Drive" without specific county data, USNM 93824–28.

Specimens which are regarded as intermediate in pattern between *jeffersoni* and *monticola* are as follows:

Carroll County: Cumberland Knob, near Galax, USNM 101824.
Floyd County: Rocky Knob Park, 6 miles southeast of Floyd on the Blue Ridge Parkway, USNM 124514–19.

Salamanders which I refer to *D. m. monticola* Dunn have been seen from a number of localities in the following counties in western Virginia: Alleghany, Augusta, Bath, Botetourt, Craig, Giles, Grayson, Highland, Montgomery, Rockbridge, Rockingham, Smyth, Tazewell, Washington, and Wise.

Certain of the preceding counties, such as Rockbridge, which extend across the Shenandoah Valley, are inhabited by both subspecies, one on the eastern edge, the other on the western.

Name.—I take considerable pleasure in associating with this distinctive Virginia salamander the name of Thomas Jefferson, a naturalist of note in his day as well as statesman and educator. Long a resident of Albemarle County, Jefferson almost certainly knew this species, which I have found close to his home.

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EXPLANATION OF PLATE

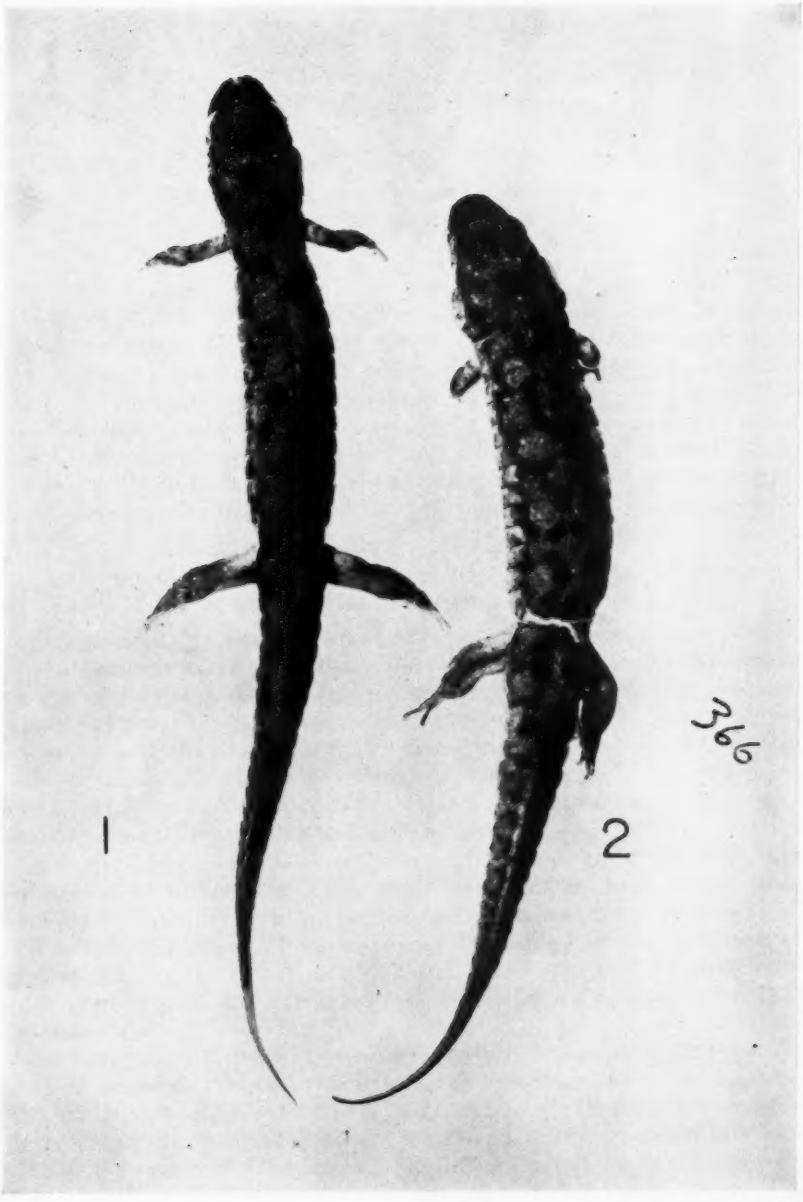
PLATE 17

FIG. 1. *Desmognathus monticola jeffersoni*, n. subsp., dorsal view of female holotype, USNM 126891, Crozet, Albemarle Co., Va.

FIG. 2. *Desmognathus monticola monticola* Dunn, dorsal view of a male, RLH 366, Longdale, Alleghany Co., Va., which agrees fairly well with the type specimen of *monticola* from Brevard, N. C.

(Photographs courtesy of Dr. Doris M. Cochran, U. S. Nat. Mus.).

PLATE 17



A NEW SUBSPECIES OF CYCLOPOID COPEPOD FROM WOOSTER, OHIO

By Harry C. Yeatman*

Department of Zoology, University of North Carolina
Chapel Hill, North Carolina

Plate 18

On April 10, 1947, Mr. Andrew Weaver and Miss Elizabeth Sgourakis of the Department of Entomology, Ohio Agricultural Experimental Station, Wooster, Ohio, found in a shallow temporary pasture pool at Wooster several examples of a cyclopoid copepod that defied identification because of the unusual armature of the fifth feet. The collection also contained *Cyclops bicuspidatus*, *Cyclops vernalis*, *Macrocyclops fuscus*, and three copepods that were apparently *Cyclops venustoides*. Mr. Weaver has sent the unidentified copepods to this laboratory for study, and they were found to be sufficiently different from *C. venustoides* to be considered an undescribed subspecies. He has asked that I name and describe them.

Cyclops venustoides bispinosus new subspecies

Body length of female varying from 1.60 to 1.90 mm. (males averaging smaller). General appearance much like that of *Cyclops venustoides* Coker, postero-lateral margins of the fourth thoracic segment not being produced. Surface of the abdominal segments densely covered with minute irregular ridges as in *Cyclops venustus* Norman and Scott, and thus differing from *C. venustoides* which often lacks these ridges. Posterior margins of abdominal segments (except the last which has tiny spinules on its posterior margin) conspicuously serrated. As in *C. venustoides* and *C. venustus*, the anal plate extends far back.

Quotient of length into width of the furcal rami is about 0.25 to 0.275; thus they are about four times longer than wide. The small lateral seta attached about three-fourths of the distance from base of ramus. Inner margins of the furcal rami with tufts of short hairs. Relative terminal furcal setal lengths, innermost to outermost, giving ratios such as 1.66:7.8:5.5:1 and 2.0:10.4:7.5:1. Innermost terminal seta almost twice as long as the outermost and only slightly longer than the ramus.

First antennae of 12 segments, extending about to the posterior margin of the first body segment. The ninth segment bearing a short aesthete on its middle.

Swimming feet with inner and outer rami of three segments. Spine formula of the terminal segments of the exopods of these feet 2, 3, 3, 3; setal formula 4, 4, 4, 4. Thus the armature of *C. bisponosus* is like that of *C. venustoides* and differs from that of *C. venustus* which has the setal formula 5, 5, 5, 5 for the segments in question.

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Quotient of length into width of the terminal segment of the endopod of the fourth swimming feet about 0.438 to 0.45. Thus the segment is approximately 2¼ times longer than broad. Quotient of length of this segment into length of longer of its two terminal spines about 0.62 to 0.63. Outer terminal spine about 1½ times longer than inner. Setae of this segment extending beyond distal ends of terminal spines.

Fifth foot of two segments. Basal segment about twice as wide as distal one and with a seta on its outer side. Distal segment small, with an apical seta, a short inner spine, and often a *short outer spine*, both spines being attached near the apex of the segment. Males show this extra spine on the fifth foot. Mr. Weaver has found that rarely the fifth foot of both males and females will have two spines on the inner side as well as the spine on the outer side of the distal segment (fig. 8). He also finds both males and females which show one fifth foot which is typical of *C. venustoides* and the other of *C. venustoides bispinosus*.

Seminal receptacle more or less transversely oval and resembling that of *C. venustoides*.

C. venustoides bispinosus closely resembles *C. venustoides* in general body appearance, number of antennal segments, hairiness of furcal rami, armature of swimming feet, and shape of seminal receptacle. It differs from that species, and from all the species of the genus Cyclops, chiefly in the armature of the fifth foot which often possesses an outer as well as an inner, short, subapical spine on the distal segment. *C. venustoides* has only a short inner subapical spine on this segment. Other and minor differences are noted: 1) *C. v. bispinosus* is larger (body length 1.60 to 1.90 mm., whereas our examples of *C. venustoides* measure only 0.82 to 1.56 mm.); 2) Irregular ridges are present on surface of abdominal segments of *C. v. bispinosus*, these are often absent in *C. venustoides;* 3) The innermost terminal furcal seta is slightly shorter than the ramus in *C. venustoides* and slightly longer than the ramus in *C. v. bispinosus;* 4) this seta is over twice as long as the outermost in *C. venustoides* and usually not quite twice as long as the outermost in *C. v. bispinosus*.

C. v. bispinosus has been found only at Wooster, Ohio. Mr. Weaver reports that it was comparatively scarce in the collection made on April 10, 1947, but by the second week in May, it was as common as *C. vernalis*.

The fifth foot of species within the genus Cyclops is occasionally abnormal. *C. viridis* (Jurine) ordinarily possesses only an inner spine and an apical seta on the distal segment, but Alverdes (1920) has reported examples with fifth feet much like those of *C. v. bispinosus*. If only one example of this form had been collected, it might have been regarded as *C. venustoides* with an abnormal fifth foot. Because it occurs in abundance usually with a notable structural feature possessed by no other copepod of the genus and with other minor differences, it must be considered a distinct subspecies of *C. venustoides*.

The typical *C. venustoides* fails to show this extra spine on the fifth foot even when reared under various controlled experimental conditions. Mr. Weaver has found and sent me examples of *C. venustoides* from Wooster which are practically identical with *C. v. bispinosus* except for the difference in the fifth foot. Since he

found intergrades that show one fifth foot like that of *C. venustoides* and the other like that of *C. v. bispinosus*, it is best to consider *bispinosus* only a subspecies. Mr. Weaver is experimenting with the effects of temperature on the presence or absence of the extra spine on the fifth foot, and hopes to attempt breeding experiments with *C. venustoides* and *C. v. bispinosus* in an attempt to clarify the taxonomic status of the latter.

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EXPLANATION OF PLATE

PLATE 18

FIGS. 1–8. *Cyclops venustoides bispinosus* n. subsp., from Wooster, Ohio.

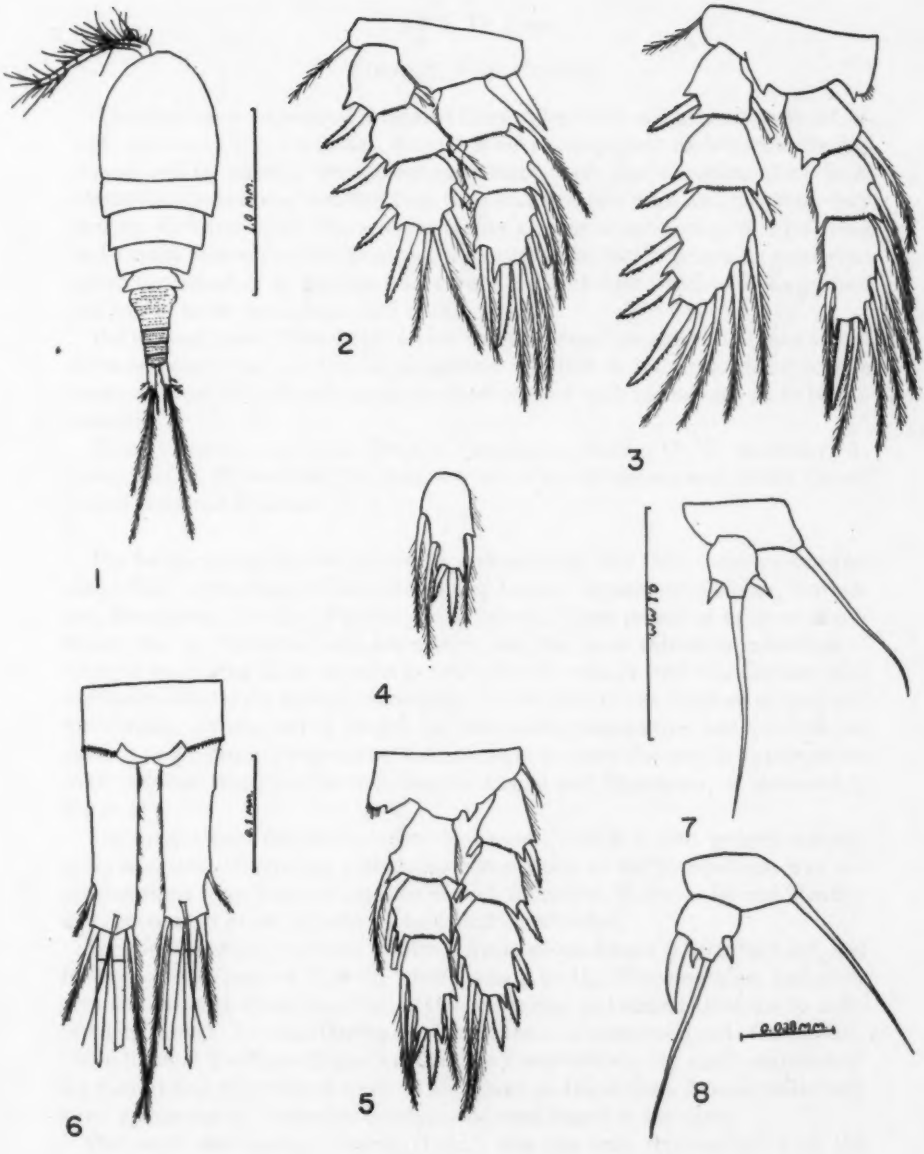
1. Adult female.
2. First swimming foot (right).
3. Second swimming foot (right) (third swimming foot has same armature as the second).
4. Terminal segment of endopod of fourth swimming foot.
5. Fourth swimming foot (left).
6. Last abdominal segment and furcal rami.

(2–6 drawn to same scale)

7. Fifth foot.
8. Abnormal fifth foot of a female (from a drawing made by Mr. Weaver).

All figures are camera lucida drawings.

PLATE 18



FAUNAL ABUNDANCE CYCLES IN A SMALL CREPUSCULAR CAVE

By J. D. Ives

Pinebluff, North Carolina

The small cave considered is located three miles south of Carson-Newman College, Jefferson City, Tennessee. Since it is never completely dark during the day it may well be called a crepuscular or twilight cave. The entrance, which faces northward, is two and one-half feet wide and five feet high and descends very sharply for twelve feet where it opens into a room about twenty-five feet long and twenty feet wide. The height of the cave varies but in general is somewhat above the height of an average man except for a crevasse which extends upward ten feet or more beyond the roof of the cave.

For several years (1944–1949) a visit was made each month to the cave to observe especially the number of mosquitoes and flies in the cave. Records were made of other animals only as far as they were of such abundance as to be noticeable.

Thanks are due to E. A. Chapin, Remington Kellog, C. W. Sabrosky, A. Stone, and H. H. Swift for the identification of the specimens sent to the United States National Museum.

The female mosquitoes of *Anopheles punctipennis* (Say) and *Culex quinquefasciatus* (Say) were found in the cave during August, September, October, November, December, January, February, and March. Their period of greatest abundance was in November and December, but the more extensive collecting of mosquitoes during those months is probably the reason why the January and February collections showed so marked a reduction in the number of recorded individuals. By the end of March all hibernating mosquitoes had vacated the cave. A new mosquito population did not begin to enter the cave in any numbers until October, though a few were seen in August and September, as indicated in Table 1.

The fungus gnat *Rhymosia inflata* (Johansen), which in size, general appearance, and time of greatest abundance corresponds to the mosquitoes, was not distinguished from them at the time of each collection. However its real identity was determined in the laboratory later and so recorded.

The flies *Oecothea fenestralis* (Fallen), *Amoebaleria defessa* (Osten Sacken), and *Heleomyza brachypterna* (Loew), which belong to the Heleomyzidae, had their period of greatest abundance during the late spring and summer months, as indicated in Table 1. At times during this period the collection consisted of more than 700 individual flies for a single day. *Oecothea fenestralis* was the most abundant of the flies. It was nearly three times as abundant as *Amoebaleria defessa*, while only a few specimens of *Heleomyza brachypterna* were found in the cave.

The moth *Scoliopteryx libatrix* (Linn.) was the only representative of the Lepidoptera that was found. Its period of abundance was about the same as that of the mosquitoes. It evidently came into the cave merely to hibernate. However,

in the spring it apparently remained in the cave somewhat longer than did the mosquitoes, and it also came into the cave later in the fall. Records were made of its occurrence in the cave in September, October, November, December, January, February, March, and April. The largest number at any one time was seventeen for January. Once settled on the roof of the cave they spent practically the entire winter in the same place. One could very easily pick them off the roof during the winter months since they were not so easily disturbed as were most of the mosquitoes.

Camel crickets, *Ceuthophilus* sp., representative of the Orthoptera, were seen during September, October, November, December, January, February, March, April, and July. Though no special study was made of this group, their greatest abundance as recorded was in October, March, and April.

TABLE I

Faunal abundance cycles in a small crepuscular cave

	ORTHOPTERA	LEPIDOPTERA	DIPTERA		CAUDATA
			Mosquitoes	Flies	
Jan.	X*	XX	C	C	X
Feb.	X	X	C	C	XX
Mar.	XX	X	C	C	
Apr.	XXX	X		C	X
May				CCCCC	X
June				CCCCCCCCC	
July	X		C	CCCCCCCC	X
Aug.			C	CCCCCC	X
Sept.	X	X	C	CCCC	X
Oct.	XX	XX	C	C	X
Nov.	X	XX	CCCCC	C	
Dec.	X	X	CCC		

* Number of individuals: X = 1–10; XX = 11–20, etc.; C = 1–100; CC = 101–200, etc.

Eurycea lucifuga (Raf.) was the outstanding salamander found in the cave, with its greatest recorded abundance occurring in February.

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INDICATOR CONSTANTS IN SOLUTIONS CONTAINING ORGANIC SOLVENTS

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In general the investigations concerning the so-called "solvent effect" in the colorimetric determination of *pH* have been lacking in careful measurement of *pH*, control of ionic strength, and careful analysis of the solution. However, Kolthoff (1, 2) and co-workers have made a careful study of the effect of methanol and ethanol on the dissociation constants of indicators. Masi and Knight (3) have determined indicator constants for thymol blue (acid range) and bromthymol blue in water, water-ethanol, and water-glycol mixtures.

This is a report on the indicator constants of phenol red, bromcresol green, and bromphenol blue in the same solvent mixtures as used by Masi and Knight (3). Hydrogen-ion activities were determined electrometrically in citrate and phosphate buffers in the various solvent mixtures. The activity measurements were combined with spectrophotometric data to calculate the apparent dissociation constant of the indicators in each of the solvents.

Theoretical

The indicator constant may be calculated by the expression,

$$pK_{In} = pa_{H^+} - \log \frac{x}{1 - x} \tag{1}$$

in which pK_{In} is the negative logarithm of the apparent indicator constant, and x is the fraction of the indicator in the basic form. x is determined from spectrophotometric data and Masi and Knight (3) have calculated pa_{H^+} from EMF measurements on the silver-silver chloride electrode combined with the hydrogen electrode. At 25°,

$$-\log a_{H^+} = \frac{E - E^\circ}{0.05915} + \log C_{Cl^-} - \frac{A\sqrt{\mu}}{1 + aB\sqrt{\mu}} + C' \tag{2}$$

$$+ \log \frac{d_0}{d + 0.001\,(M_1 - M_2)\,C}$$

where C_{Cl^-} is the molarity of the chloride ion, A and B are Debye-Huckel constants, a is the ion-size parameter, μ is the ionic strength, C' is the "salting out" constant, d_0 and d are the densities of the solvent and the solution, M_2 is the molecular weight of the chloride ion, and M_1 the mean molecular weight of the solvent. Hydrogen-ion activities calculated by (2) are expressed in moles per liter. In cells without liquid junction the variable contribution of the liquid junction potential to E and E° is eliminated. Hence pa_{H^+} values calculated from

(2) and pK_{I_a} values from (1) should be more directly comparable in the different solvents employed. However, the $E°$ values of equation (2) are referred to the standard state of unit activity in the solvent used. Hence, a change of solvents means a different reference state that does not allow direct comparison with complete confidence. The method used here seems to be the best that can be done unless someone solves the problem of calculating absolute ionic activities.

EXPERIMENTAL

Potential measurements were made of the cell

$$Pt, H_2 \mid Solution \mid AgCl, Ag$$

at 25° in solutions of hydrochloric acid in water, in 10 and 20% by weight ethanol-water mixtures, and in 20 and 40% by weight ethylene glycol-water mixtures. Potential measurements were also made on hydrochloric acid-citrate buffers and on phosphate buffers in the above five solvents. A constant amount of potassium chloride was added to each phosphate buffer.

After electromotive force measurements had been made on a solution, phenol red, bromcresol green, or bromphenol blue was added and the fraction of the indicator in the basic form was determined spectrophotometrically.

MATERIALS

The solvents, water, ethanol, ethylene glycol, and solutions of hydrochloric acid, monopotassium citrate, monopotassium phosphate, potassium chloride, and sodium hydroxide were prepared as previously described (3).

Indicator Solutions. The indicators obtained from Coleman and Bell were used without further purification. One-tenth percent stock solutions were prepared according to the directions of Clark (4).

Hydrogen Gas. Commercial tank hydrogen was purified by passage in succession through soda-lime, "Drierite," heated copper, soda-lime, and "Drierite."

Solutions for Measurements. Five series of solutions of different solvent composition were prepared as follows: Series I, water; Series II, 10% ethanol-water; Series III, 20% ethanol-water; Series IV, 20% ethylene glycol-water; Series V, 40% ethylene glycol-water.

The solutions in each series were sub-classified into three series as shown below:

GENERAL p_{aH}	RANGE	INDICATOR	BUFFER SYSTEM
A.	3.0–4.6	Bromphenol blue	3.0–3.6 HCl-citrate 3.8–4.6 NaOH-citrate (excess)
B.	3.8–5.4	Bromcresol green	3.8–5.0 NaOH-citrate (excess) 5.0–5.4 citrate-NaOH (excess)
C.	6.8–8.0	Phenol red	6.8–8.0 NaOH-phosphate

The method of preparing the solutions of known composition and ionic strength, of preparing electrodes, and of measuring the emf. of the cells has been previously described (3).

Spectrophotometric Measurements.

A Coleman Model 10 S double monochromator spectrophotometer equipped with a 5 mμ slit was used to measure the transmittance of the indicator solutions in some cases. At other times, a Beckman quartz spectrophotometer was used. No sensible difference was observed in the transmittance measurement when the same solution was examined in the two instruments. Transmittance measurements were at a wave length of 592 mμ for bromphenol blue, at 617 mμ for bromcresol green, and at 558 mμ for phenol red, the points of minimum transmittance.

In agreement with Brode (5), it was found that the wave length of minimum transmittance did not change with *pH*. The addition of the organic solvent did not change the wave length of minimum transmittance but did change the intensity of the transmitted light, thereby necessitating its presence in the spectrophotometric reference solution.

Two-tenths of a milliliter (±0.002) of indicator solution were added to 20.0 milliliters (±0.02) of a solution on which an electromotive force measurement had been made. The ratio of the extinction value of the solution under examination to the extinction value of the indicator in its completely basic form is equal to x of equation (1) from which pK_{In} is calculated. In all cases except those involving bromphenol blue in glycol solutions, the full basic color was developed in 0.1 N NaOH solution containing the appropriate solvent. In the latter case, 0.01 N NaOH solution was used to develop the full color because of an appreciable fading of the color of the indicator in the glycol solution.

CALCULATIONS AND RESULTS

The ionic strengths of the various buffer solutions were calculated as has been described in a paper (3). *paH* values were calculated by equation (2) using the calculated ionic strengths, the measured electromotive forces and the constants listed in Table I. pK_{In} values were then obtained from equation 1.

Tables II and III show the experimental results of the 20% ethanol series for bromcresol green and phenol red, respectively. The values for concentration, ionic strength, electromotive force, moles of NaOH (Y) per mole of buffer salt, *paH* and pK_{In} are typical of all of the five series of solutions.

Table II shows values for citrate-sodium hydroxide buffers used with bromcresol green in 20% ethanol-water.

Table III shows values for phosphate-sodium hydroxide buffers used with phenol red.

The mean values of the apparent indicator constants of bromphenol blue, bromcresol green and phenol red together with the average deviation from the mean are recorded in Table IV for the five series of solutions. Table V shows the effect of the solvent on the *paH* of the buffer solutions and on the constants of

TABLE I

Constants for equation 2

	WATER	10% EtOH	20% EtOH	20% GLYCOL	40% GLYCOL
Series	I	II	III	IV	V
E_c° volts	.22154	.21343	0.2560	.21137	.19968
A_c	.5089	.5701	.6457	.5701	.6515
B × 10^{-8}	.3286	.3414	.3558	.3413	.3568
D	78.54	72.8	67.0	72.8	66.6
d_0	.99707	.98038	.96640	1.0230	1.0490
M_1	18.0	19.2	20.5	21.00	25.16
$(M_1 - M_2)$ .001	−.0175	−.0163	−0.150	−.0145	−.0104
Series	I A	II A	III A	IV A	V A
a × 10^8	4.3	5.8	5.6	3.7	4.3
C'	.13	.135	.14	*	.156
Series	I B	II B	III B	IV B	V B
a × 10^8	4.3	5.8	5.6	3.7	4.3
C'	.13	.135	.14	*	.156
Series	I C	II C	III C	IV C	V C
a × 10^8	4.5	4.5	4.5	4.5	4.5
C'	Varying values				

* Cl^- concentration .005m to .012m $C' = -.60$
Cl^- concentration .012m to .025m $C' = -.22$

TABLE II

The indicator constant of bromcresol green in 20% ethanol solution

SOL. NO.	μ	pH	pK_{In}	X	Y	E (COR.)
1	0.0500	4.178	5.18	0.0914	0.0400	0.57672
2	.0540	4.383	5.17	.1401	.1800	.58902
3	.0513	4.595	5.15	.2195	.3261	.60144
4	.0509	4.803	5.13	.3205	.4737	.61369
5	.0495	5.016	5.14	.4354	.6289	.62624
6	.0511	5.233	5.16	.5444	.7842	.63910
7	.0516	5.429	5.20	.6290	.9344	.65070
8	.0507	5.691	5.26	.7306	1.0848	.66620
9	.0502	5.906	5.33*	.7886	1.2193	.67948
Ave.			5.17			
Ave. Dev.			±.03			

* Out of range, not included in average.

the three indicators. In this table, values of *paH*, pK_{In}, and D from aqueous solutions are used as references for computing Δ values. Thus, Δ *paH* refers to the change in *paH* due solely to the addition of the organic solvent, other factors remaining constant.

An inspection of Table V shows the following effects of the solvent on the

TABLE III

The indicator constant of phenol red in 20% ethanol solution

SOL. NO.	μ	p_{H^+}	pK_{In}	x	y	E (cor.)
1	0.0537	7.311	8.17	0.1270	0.4713	0.76290
2	.0536	7.527	8.19	.1796	.5905	.77554
3	.0528	7.772	8.15	.2918	.7238	.78985
4	.0513	7.913	8.18	.3501	.7874	.79813
5	.0506	7.964	8.18	.3811	.8539	.80109
6	.0507	8.236	8.21	.5236	.9034	.81718
7	.0502	8.347	8.16	.6044	.9371	.82369
Ave.			8.18			
Ave. Dev.			±.01			

TABLE IV

Indicator constants in water-organic solvents

SOLVENT	pK_{In} BROMPHENOL BLUE	pK_{In} BROMCRESOL GREEN	pK_{In} PHENOL RED
Water	4.01 ± 0.02	4.76 ± 0.04	7.81 ± 0.01
10% Ethanol	4.05 ± 0.04	4.92 ± 0.03	7.97 ± 0.02
20% Ethanol	4.20 ± 0.03	5.17 ± 0.03	8.18 ± 0.02
20% Glycol	4.04 ± 0.01	4.83 ± 0.02	7.98 ± 0.03
40% Glycol	4.23 ± 0.01	5.07 ± 0.04	8.12 ± 0.02

TABLE V

Change in pK_{In} due to the presence of organic solvents

	ΔD	Δp_aH	ΔpK_{In}
Bromphenol blue (Citrate buffers. μ = approx. 0.05)			
10% Ethanol	5.7	0.20	0.06 ± 0.04
20% Glycol	5.7	0.20	0.03 ± 0.01
20% Ethanol	11.5	0.37	0.19 ± 0.05
40% Glycol	11.9	0.46	0.22 ± 0.02
Bromcresol green (Citrate buffers. μ = approx. 0.05)			
10% Ethanol	5.7	0.20	0.16 ± 0.03
20% Glycol	5.7	0.20	0.07 ± 0.04
20% Ethanol	11.5	0.37	0.42 ± 0.03
40% Glycol	11.9	0.48	0.31 ± 0.03
Phenol red (Phosphate buffers. μ = approx. 0.05)			
10% Ethanol	5.7	0.23	0.15 ± 0.02
20% Glycol	5.7	0.54	0.17 ± 0.03
20% Ethanol	11.5	0.47	0.36 ± 0.02
40% Glycol	11.9	0.76	0.31 ± 0.03

paH values as compared to pure water: (a) In low concentrations of glycol, the effect on *paH* is the same as for ethanol of the same dielectric constant; (b) in high (40%) glycol solutions, the effect on the *paH* is greater than for ethanol of the same dielectric constant; (c) the effect on *paH* in phosphate buffers is in the same direction as previously stated. However, the effect is much greater in phosphate buffers than in citrate buffers.

The effect of the organic solvent on the pK_{In} values of the three indicators may be summed up as follows: (a) Bromphenol blue shows little change in pK_{In} in low organic solvent concentrations but as the concentration of organic solvent is increased, the increase in pK_{In}, and hence the solvent correction, becomes noticeable; (b) bromcresol green shows a noticeable solvent correction, particularly in the case of ethanol. While ethanol has a smaller effect on *paH*, it has a larger effect on pK_{In}; (c) phenol red has an appreciable solvent correction but there is not a great deal of difference between ethanol and glycol.

While it is to be expected that the addition of an organic solvent will increase the *paH* of a solution, the effect of the solvent cannot be explained solely on the change in dielectric constant. Mixed solvents of the same dielectric constant do not show the same value of *paH*. Finally, just as the solvent exerts a specific influence, the change in pK_{In} depends not only on the solvent but on the specific nature of the indicator. No general conclusions can be drawn until more data are accumulated.

SUMMARY

1. Electromotive force measurements have been made at 25° on the cell Pt, H_2 | Solution | AgCl, Ag in five solvents, water, 10% by weight ethanol-water, 20% by weight, ethanol-water, 20% by weight ethylene glycol-water, and 40% by weight ethylene glycol-water.

2. Hydrogen ion activities have been calculated from the electromotive force measurements and used, along with spectrophotometric measurements, to determine the apparent indicator constants of bromphenol red, bromcresol green, and phenol red in the five solvents.

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STUDIES IN THE GENUS CHYTRIDIUM, WITH OBSERVATIONS ON A SEXUALLY REPRODUCING SPECIES[1]

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Plates 19–21 and Two Text Figures

The parasite of *Vaucheria* studied here is of special interest because it is the first species of the one-hundred year old genus *Chytridium*, as currently defined, in which sexuality is fairly certainly reported.

This fungus, *Chytridium sexuale* sp. nov., which flourishes as a parasite and is only weakly saprophytic, was found in early June on *Vaucheria geminata* in a well-shaded, small, sluggish stream in the coastal plain of North Carolina.

Both the host and parasite grew well in a cold room which had been adapted for stocking pure cultures of algae (100–150 foot candles of fluorescent and incandescent light for 8 hrs. daily; temperature 10° C., increasing to 18° C. with the lights). It was necessary to use these culture conditions because the host, and consequently its parasite, failed to grow and soon degenerated under ordinary room conditions during the summer. Although the chytrid grew and reproduced abundantly in the cold room environment (figs. 1 & 2), complete development of an individual required up to about one week. Furthermore, either the microscope stage had to be cooled for prolonged observations at room temperature or else only intermittent observations on material growing in the cold room could be made.

No attempt was made to get the material into pure, host-parasite culture. In fact, two strict saprophytes, *Entophlyctis* sp. and *Chytridium* sp., as well as *Woronina glomerata*, were also present.

Chytridium sexuale sp. nov.

Fungus parasiticus et infirme saprophyticus in *Vaucheria geminata*. Sporangio orto ex apice germinatae zoosporae, ferme obpyriformi, 12–43μ longo, 6–21μ lato, plerumque 30–40 x 14–19μ; curvato, raro cylindrico vel leviter helicoideo; pariete hyalino, levi; disjuncto operculo 4.9–15.6μ in diam., plerumque 11–12.5μ. Zoosporis globosis, 3.2–4.5μ in diam., cum excentrico refractivo globulo 1.2–4.6μ in diam.; flagello circa 17μ longo; emergentibus separatim et quietis punctum temporis. Apophyside 3.2–9.2μ in diam., plerumque 6.5–8μ; systema rhizoidorum nulla. Spora perdurante sexualiter formata, 11.3–14μ in diam., cum largo excentrico refractivo globulo, pariete verruculoso 0.6–1.1μ crasso. Extramatricali parte feminei thalli 4.3–7.8μ alta; masculina cellula efficiente; spora perdurante intramatricali; germinatione incomperta.

Sporangium, usually on an inconspicuous stalk up to 2.2μ long, arising as an

[1] The author wishes to acknowledge his appreciation for the counsel of Dr. J. N. Couch in the preparation of this study and to thank Mrs. Alma H. Beers for preparing the Latin diagnosis.

outgrowth of the distal end of the germinated zoospore, which is 2.5–4.0μ wide and is the basal part of the sporangium; rarely perpendicular to the host wall and with radial symmetry, but usually somewhat inclined and bent slightly or through nearly a 180° angle and bilaterally symmetrical; rarely twisted or helicoidal; essentially obpyriform or spatulate and usually with a slight bulge in all directions between the zoospore and broadest part, and gibbous distally when bent over; 12–43μ long x 6–21μ wide (mostly 30–40 x 14–19μ); wall colorless, smooth, remaining intact after discharge of zoospores; detached operculum 4.9–15.6μ in diameter (mostly 11.0–12.5μ). Motile cells spherical, 3.2–4.5μ in diameter, with an excentric, colorless, highly refractive globule 1.2–1.6μ in diameter, and a flagellum about 17μ long; emerging fully cleaved, separate and spherical, through a terminal, circular orifice when the operculum is completely detached, or through a slit when the operculum is only partly detached, lying quiescent for about one minute in a loose cluster while unwrapping their flagella, and then rapidly dispersing, some of them remaining within the sporangium and emerging one to a few at a time; swimming cell with a posteriorly attached and directed flagellum. Penetration tube of the encysted motile cell when passing through the host wall is 0.7–1.3μ wide, reduced abruptly to 0.3–0.9μ in width within the host protoplasm; distal end swelling to form the spherical apophysis, 3.2–9.2μ in diameter (mostly 6.5–8.0μ) with a smooth wall; no rhizoidal system. Resting body essentially spherical, 11.3–14.0μ in diameter, protoplast 9.5–12.0μ wide with a large, excentric, colorless, highly refractive globule surrounded by many much smaller ones, and a wall 0.6–1.1μ thick, including the covering of numerous, short, blunt warts; sexually formed; female thallus indistinguishable from young sporangial thallus, the epibiotic part 4.3–7.8μ high, with the cyst portion inconspicuously to considerably expanded distally; contents of the encysted male cell, which is 3.2–3.9μ in diameter, passing through a germ tube into the epibiotic, expanded part of the female thallus; the fusion protoplasm moving into the endobiotic, swollen tip of the germ tube, where the resting body or zygote matures; germination not observed.

Parasitic and weakly saprophytic on *Vaucheria geminata* DC.; from a well-shaded, small, sluggish stream running into the Neuse River, about 2 miles north of Fort Barnwell, Craven County, North Carolina; June 1951. Preserved material of the type collection is deposited in the herbarium of the University of North Carolina.

The genus *Chytridium* is understood as including all monocentric, eucarpic chytrids with an endobiotic vegetative system and resting body and with an epibiotic, operculate sporangium which forms by the expansion of the germinated zoospore. *Chytridium sexuale* agrees in these respects with all other species of the genus except for *C. curvatum*, "where the tip of the short sub-sporangial stalk evidently does not penetrate beyond the thin layer of gelatinous material surrounding the *Stigeoclonium* filament" (Sparrow, 1943). The sporangial thallus of the new species is unique in its combination of characteristics[2]. Other species also described

[2] After this paper had been sent to press, a reprint of an article by Fott (1951) was brought to my attention. It contains the description of a new species, *Chytridium Cejpii*, which has a sporangial thallus much like that in *C. sexuale*. Fott did not find resting body formation for *C. Cejpii*. Both chytrids parasitize *Vaucheria*. They differ most conspicuously in that the sporangium of *C. Cejpii* is perpendicular to the host wall and apparently has radial

as having a spherical apophysis and no rhizoids are *C. brevipes* Braun, *C. inflatum* Sparrow and *C. Scherffelii* (Scherffel) Sparrow. In the development and shape of the sporangium it is somewhat like *C. curvatum* Sparrow and *C. versatile* Scherffel. This fungus differs from all other species of *Chytridium* by the sexual formation of the resting bodies, and by the warted structure of their walls.

The fungus is strongly parasitic, with young sporangial and resting body thalli developing concurrently nearly to the active tips of *Vaucheria* filaments. Mature stages in development are not found at the tips, and this is undoubtedly due to the fact that the host outgrows its parasite. Fixed and stained material shows mitotic figures in the host's filaments at a time when resting bodies and sporangia of the parasite have matured.

Damage to the host is relatively minor, plasmolysis occurring only on heavily infected filaments. The first sign of a moribund condition is the appearance of collapsed, dark green and sometimes clumped chloroplasts moving along with the flowing cytoplasm. Callus formation, when observable, is restricted to the area of the penetration tube for sporangial thalli but often results in a thin or thick coating around the resting body (fig. 26).

The germ tube of the encysted zoospore or female motile cell penetrates the host wall and becomes abruptly reduced in width by about one-half when growing in the thin peripheral layer of host protoplasm. The apophysis has its beginning when the distal end of the narrowed germ tube begins to swell, with tiny lipoidal globules appearing inside. At the same time the encysted portion, which retains its conspicuous globule, becomes ovoid, elliptical or oblong, and the distal tip begins to expand (figs. 12 & 13); thus, the old encysted motile cell becomes the incipient sporangium or the female receptor on which the male cell encysts, as the case may be.

The apophysis and narrow germ tube are usually displaced by the streaming host protoplasm (figs. 6, 7, & 13). One of the lipoidal globules of the apophysis at an early stage becomes larger than the rest, which condition is maintained throughout the expansion of the apophysis. This is also the situation in resting body formation. It is clearly seen that rhizoids never form; the endobiotic system is merely the germination tube with its swollen tip. The enlarged apophysis causes a conspicuous dent in the vacuolar membrane of the host's large central vacuole, which is never penetrated (fig. 16). In some cases the apophysis remains filled with protoplasmic contents while the sporangium forms, matures, and discharges its zoospores, but usually its contents empty into the sporangium before the spore initials are formed (fig. 16b).

Sporangia show considerable variation in their shapes and in their orientation to the host (figs. 1 & 2). All, however, are diagnosed as essentially obpyriform or spatulate. There is usually a slight bulge in all directions between the basal cyst and the broadest, distal part, and the sporangium is hump-backed (distally gib-

symmetry, whereas in *C. sexuale* the sporangium is characteristically bent over and is bilaterally symmetrical. Although the two chytrids in question may really be the same species, at present there is not enough information to justify considering them identical.

bous) when it is bent. The three fundamental growth forms are illustrated in text fig. 1. The few sporangia which I observed to be radially symmetrical were perpendicular to the host wall (A). One would expect to find them inclined, too, if they grew out in a regular manner from an encysted zoospore which had penetrated at an angle (fig. 12, right). Most sporangia are bilaterally symmetrical (B) with the curved body bent through nearly a 180° angle in some cases. A few sporangia are twisted in a spiral manner (C) and may be either perpendicular or inclined to the host wall.

The sporangium is vacuolate from incipience to maturity (figs. 12–16). Although the vacuoles are frequently difficult to delimit, there is indirect evidence of their abundance when sporangia treated with salt solution are observed to plasmolyze considerably in all stages of development except in the mature sporangium, where the spores merely round up in an irregular fashion, without separation of the entire mass from the sporangium wall.

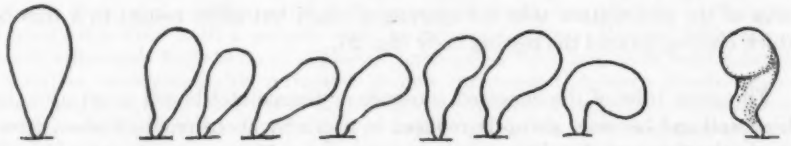

FIG. 1. Symmetry and shapes of sporangia in *Chytridium sexuale*. A, radially symmetrical sporangium; B, bilaterally symmetrical sporangia; C, slightly helicoidal sporangium. (× 500)

When the sporangium is full-sized, the lipoidal globules, some of which are always larger than those of the zoospores, are in the "cluster stage," that is, the globules are more or less centrally disposed, leaving an irregular margin of clear, homogeneous or often vacuolate cytoplasm (figs. 6a & 16a). The globules subsequently become very finely divided and evenly distributed throughout the vacuolated cytoplasm (fig. 16b). Due to the particulate nature of the lipoidal material in this "dispersion stage" the cytoplasm has a more or less granular appearance. There is then a confluence, following no regular pattern, of the lipoidal material into rather large, similarly sized globules, one for each zoospore initial (fig. 16c). The outlines of the cleaved zoospores are not especially clear even just before dehiscence, but there is evidence from stained material that cleavage may start even considerably before the complete confluence of the tiny lipoid globules; in other words, that at the time of cleavage there are several globules for each zoospore initial.

Although no special wall or plug could be seen at the base of the fully enlarged sporangium, it is certain that the protoplast is in some way effectively isolated from the apophysis; for when nearly mature sporangia are broken off from the host, they continue development and discharge their zoospores normally. The short stalk of the detached sporangium appears homogeneous and highly refractive.

The complete emptying of the zoospores from a sporangium takes from about three minutes to over one hour. This wide variation in length of time is governed in large part by whether the zoospores emerge through a circular orifice after the complete detachment of an operculum (figs. 3 & 18), in which case the emptying is rather rapid, or through a wide to narrow slit which results from an incompletely formed or detached operculum (figs. 4, 16, & 19). Several dozen discharging sporangia have been observed with the material both in hanging drop and under cover slip, and both formations are apparently normal and of about equal frequency.

There is, however, only one basic pattern of emergence. About two-thirds of the complement come out initially, one to several at a time, with each zoospore separate and spherical (fig. 16d); these lie quiescent in a loose cluster for one to several minutes while unwrapping their flagella, and then disperse more or less simultaneously (fig. 16e). The zoospores left within the sporangium emerge singly or by twos or threes, each with its flagellum wrapped around its body at first (fig. 16e) but later ones with the flagellum already extended (figs. 16f & 3). Often the aperture is so narrow that the zoospores left in the sporangium can escape only in an amoeboid manner and pull their flagella free (figs. 16f & 4).

The spherical body of the zoospore contains a conspicuous, excentric lipoidal globule and one to a few, small, clear spaces within the pale, homogeneous cytoplasm. The flagellum on the swimming zoospore is posteriorly attached and directed. When otherwise at rest the flagellum sometimes shows a lashing endpiece. The lipoid globule is always excentric and, in relation to the point of attachment of the flagellum, maintains a rather fixed position, although this position varies from spore to spore within the same sporangium. After the swimming zoospores are killed with the fumes of a 1% solution of osmium tetroxide and stained lightly by adding a minute amount of crystal violet, the nucleus and nuclear cap, along with the lipoid globule, are clearly seen within the zoospore body (figs. 5 & 17).

Attempts to discover a morphological differentiation between motile cells were inconclusive or futile. There are, however, two types of behavior exhibited by them; that is, the motile cell may encyst on (1) the host wall (fig. 11), or (2) the epibiotic, distally expanded part of the female thallus (fig. 20). In the first case, either a sporangial thallus or a female thallus is produced subsequently. The female thallus appears to be nothing more than a sporangial thallus with its exogenous development arrested at an early stage by the engagement of the male cell (figs. 20–23); however, there is no proof of this.

In any event the content of the encysted male cell empties through a germ tube into the distally expanding part of the female thallus (figs. 7 & 23). The combined protoplasts then move into the endobiotic swelling, increase in size, and at maturity are invested with a warted and apparently single-layered wall (figs. 9 & 25).

In all of the other described species of *Chytridium* resting bodies are ostensibly asexually formed. In view of the type of sexuality reported here for *C. sexuale*, Sparrow's observation (1936) on *C. Lagenaria* of "a curious conjugation of en-

cysted zoospores with young thalli developing in pure water" is especially interesting. The thalli resulting from these fusions did not produce resting bodies, but rather a rejuvenated vegetative growth, prolonged for nearly a week, "in contrast to ordinary germinating zoospores, which under the same conditions disintegrated within twelve hours" (Sparrow, 1943).

The striking difference in size of the resting body and the apophysis between *Chytridium stellatum* (compare "e" with "r" in fig. 28) and *C. sexuale* (compare fig. 6 with fig. 9) instigated a more accurate comparison of all species for which the diameter of the protoplasts of the apophysis and resting body could be calculated or estimated. The protoplasts rather than the entire structures were used because they show a close correspondence of the maximum diameters within each of the asexual species. This is summarized in text fig. 2. Projected above the micron scale are bars which cover the actual size ranges. For example, in *C. sexuale* the apophysis protoplast measures 1.2–8.3μ in diameter and the resting body protoplast 9.5–12.0μ. It is seen that in this species with sexuality, the range of sizes of the resting body falls completely outside that of the apophysis; the smallest resting body is larger than the largest apophysis. However, in the other species, which have asexually formed resting bodies, the size range of the resting body falls within that for the apophysis; or, from another point of view, the maximum size of the resting body does not exceed the maximum size of the apophysis (except for *C. oedogonii* with a 0.2μ overlap). That the resting body in *C. sexuale* is entirely larger than the apophysis cannot, of course, be accounted for by the physical addition of the small male protoplast. Is the separation of size ranges related to sexuality? More extensive quantitative data must be obtained for sexual species and for those presumed to be asexual.

Chytridium stellatum comb. nov.

Phlyctochytrium stellatum H. E. Petersen. Bot. Tidsskrift **29:** 417, 1909; Ann. Mycologici **8:** 550, fig. XXII a, b, d and Fig. XXIII, 1910.

Sporangium extramatrical; sessile; procumbent, resting on the host wall; main body of sporangium thin-walled, arising as a lateral outgrowth from the basal part of the encysted and germinated zoospore, which becomes displaced laterally and is part of the sporangium; thin-walled part subspherical, elliptical, ovoid or somewhat irregular, 5.1–44.0μ long x 4.4–26.4μ wide; wall colorless, smooth, thin and delicate, collapsing after discharge of zoospores, except for persistent, thick-walled cyst; exit papilla, when discernible, broadly convex, variously located. Zoospores spherical, 4.2–5.5μ in diameter with an excentric, colorless, highly refractive globule, 2.2–3.3μ in diameter, and a flagellum about five times as long as the diameter of the spore body; emerging through an orifice after the detachment of an operculum, about 5μ in diameter, and forming a more or less globular mass, with individual zoospores distinct but adpressed, and lying quiescent for about one minute, while slightly parting and extending their flagella, and then rapidly dispersing, the swimming spore with a posteriorly attached and directed flagellum. Apophysis intramatrical, spherical, 6.0–15.4μ in diameter, or slightly fusiform, wall holding shape after discharge of sporangium, connected to extramatrical, encysted zoospore by old germ tube, 0.5–1.5μ in diameter; rhizoidal system coming out from base of apophysis, extensively branched, rhizoids 0.2–

1.5μ in diameter, with blunt tips, sometimes becoming extramatrical. Resting body apparently formed asexually, in intramatrical swelling; spherical, 11.0–26.4μ in diameter with protoplast 8.8–14.3μ wide, or subspherical; wall two-layered, hyaline, glistening, outer layer with numerous, prominent excrescences up to 6μ long, straight or bent, pointed, blunt or regularly to irregularly clavate; with a large, excentric, colorless, highly refractive globule surrounded by many much smaller ones; empty, extramatrical cyst remaining expanded or all but basal portion soon collapsing; germination not observed.

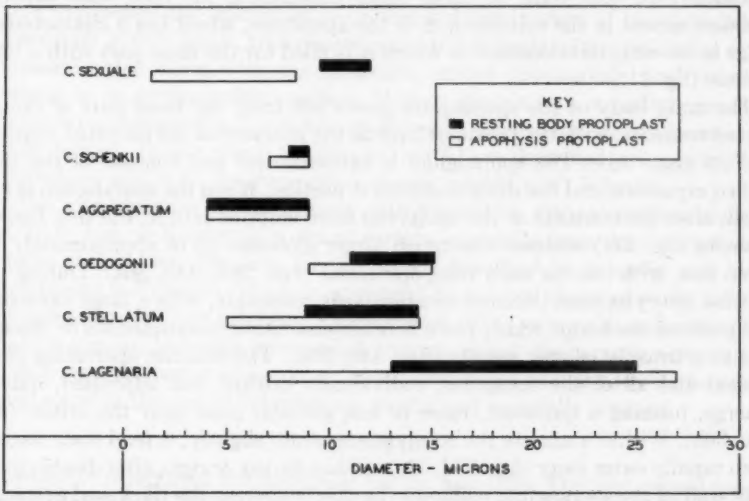

FIG. 2. Graph comparing size ranges of the resting body and apophysis protoplasts of each species of *Chytridium* for which data are obtainable. Projected above the micron scale are bars which cover the actual diameter ranges. In *C. sexuale*, the only sexual species, the gap between the range in size of the apophysis and resting body stands in marked contrast to the coincidence of ranges in size in each of the other, apparently asexual species. (For further explanation see text.)

(Measurements were calculated for *C. Schenkii* (Schenk) Scherffel from Sparrow, 1943; for *C. aggregatum* Karling from Karling, 1938; for *C. Oedogonii* Couch from Couch, 1938; and for *C. lagenaria* Schenk from Karling, 1936 and Sparrow, 1943. Measurements for only spherical structures were used. For all species, in calculating the diameter of the apophysis protoplast, an arbitrary value, 1μ, was considered for the two wall thicknesses.)

Parasitic or saprophytic on *Zygnema sp.;* spring run at Sparrow's lower pasture near Chapel Hill, Orange Co., North Carolina; January 1950.

The wall of the host is penetrated by the germ tube of the encysted zoospore (fig. 30), with occasional resistance from the host with the formation of extra wall material (fig. 35-c). According to Scherffel the intramatrical tip of the germ tube swells, forming the young apophysis, from which the rhizoids grow out. I have observed one such swollen germ tube tip without a rhizoidal outgrowth. However, although early development has not been followed through, my obser-

vations indicate that the general method of development of the intramatrical apophysis is likely to be as described by Karling (1936) for *Chytridium Lagenaria*. In some cases the germ tube is extremely long and the apophysis is formed extramatrically (fig. 34); in one case the germinated spore, the apophysis and an extensively branched rhizoidal system were developed extramatrically, only one rhizoid penetrating the host wall. The system of branched and blunt-tipped rhizoids often extends through several host cells. The rhizoids sometimes become extramatrical, with some of them again penetrating the host wall. Lipoidal globules appear in the rhizoids and in the apophysis, which has a characteristic stage in its early development in which it is filled for the most part with a large globule (fig. 31).

The main body of the sporangium grows out from the basal part of the encysted zoospore, with the proximal limit at the juncture of the encysted zoospore and its germ tube. The sporangium is extramatrical and consists of the thin-walled expansion and the thick-walled cyst portion. When the sporangium is full-sized, after the contents of the apophysis have emptied into it, the tiny lipoidal globules (fig. 33a) coalesce into much larger globules, all of approximately the same size, with one for each zoospore initial (figs. 28-s, 33b, 36a). During this process the cytoplasm becomes conspicuously vacuolate, with a large vacuole at the point of discharge, which place is otherwise either inconspicuous or discernible as a broadly convex papilla (figs. 33b, 36a). The delicate operculum is detached and all of the zoospores, individually distinct but adpressed, quickly emerge, forming a quiescent, more or less globular mass near the orifice (figs. 33c, 36b). Within a minute the zoospores separate slightly, extend their flagella, then rapidly swim away (figs. 33d, 33e). Either during or right after discharge the thin wall of the sporangium collapses (fig. 33c), whereas the thickened extramatrical cyst and the intramatrical apophysis wall remain intact (fig. 28-e).

No part of the resting body or its thallus, or of the sporangial thallus, gives a positive test with chloroiodide of zinc for cellulose.

The resting bodies are apparently formed asexually and develop along with sporangium-producing plants on the same host filament (fig. 28). Although complete development has not been followed through, it is likely that a young thallus (fig. 37) in which the contents of the encysted zoospore have emptied into the intramatrical part is an early stage in the formation of the resting body. The extramatrical cyst may either remain intact (figs. 38, 40 & 41) or all but the thickened proximal portion may collapse (figs. 37 & 39). At a later stage in development the intramatrical swelling (incipient resting body) has increased in size, contains several rather large lipoidal globules (fig. 38), and has very faint excrescences on the outer wall (fig. 39), which is the wall of the original thallus swelling. At maturity an inner wall apparently completely isolates the resting body protoplast, which contains a large lipoid globule surrounded by many much smaller ones (figs. 29 & 41). On the mature resting spore the excrescences are glistening and conspicuous (fig. 29) and are certainly the most striking feature of this species.

It has been pointed out by Sparrow (1943) that, from the intramatrical posi-

tion of the resting body, Petersen's *Phlyctochytrium stellatum*, which was described more fully by Scherffel in 1926, is clearly no *Phlyctochytrium*, and that it is possibly a form with a method of development similar to that of *Chytridium Schenkii*. Scherffel was not able to determine whether the sporangium is intra- or extramatrical, and Petersen did not record any observations about this. Although neither mentioned an operculum, they did not make particular observations on the formation of the discharge papilla and orifice. That both investigators could easily have overlooked the operculum is understandable in view of the fact that the operculum is very small, transparent, thin and delicate, often collapsing and indistinguishable along with the collapsed, empty sporangium, and frequently formed in an unobservable position. If their fungus was in reality operculate, which is my assumption, it must be considered a *Chytridium*, as the genus is presently defined. In any event, the fungus that I have described is a species new to *Chytridium* and in its development is closely related to *C. Schenkii*.

The identification of this fungus with Petersen's *Phlyctochytrium stellatum*, found by him on *Spirogyra*, is almost certain, especially from the description and figures of Scherffel. The host for the fungus described by Scherffel and for the present one is *Zygnema*. There is agreement in size and shape of parts, although the size ranges are now extended. Development of the sporangial and resting body thalli is similar, although sporangium formation and discharge is now clarified. Finally, the distinctive excrescences of the wall of the resting body in the present fungus and in the one of Scherffel are similar and especially striking.

SUMMARY

Sexuality is reported in *Chytridium* for the first time. The new, sexual species, which was found growing parasitically on *Vaucheria*, is named *Chytridium sexuale*. Its morphology and development are studied from living material. Another *Chytridium*, which grows on *Zygnema*, is studied and is considered identical with the incompletely described *Phlyctochytrium stellatum*. It is therefore named *Chytridium stellatum*. A comparison between the size ranges of the resting body and the apophysis is made for the sexual species and the presumably asexual species.

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EXPLANATION OF PLATES

All drawings and photomicrographs are of living material, except for Figs. 5 & 17. Drawings made with the aid of a Spencer camera lucida. Photomicrographs taken with an E. Leitz "Makam" attachment.

PLATE 19

Chytridium sexuale sp. nov.

FIGS. 1 & 2. × 100, using × 10 obj. Wratten "M" plates, contact prints.

FIGS. 3 & 4. × 860, using × 43 obj. DuPont 428 sheet film, enlargements × 2.

FIGS. 5–9. × 930, using × 93 oil obj. Wratten "M" plates, contact prints.

FIGS. 1 & 2. Heavily parasitized filaments of *Vaucheria*, giving a good impression of variation in sporangium shape. Mature resting bodies, *r*, appear as glistening spots inside host filaments.

FIGS. 3 & 4. Nearly empty, discharging sporangia. In fig. 3 the zoospores are swimming inside the sporangium; here also is a completely detached operculum which has remained by the orifice (cf. fig. 18). In fig. 4 is shown a discharging sporangium in which the operculum was incompletely formed and thus incompletely detached (cf. figs. 16f & 19). Note excentric position of lipoid globule in zoospore.

FIG. 5. Killed and lightly stained zoospore. The flagellum is attached beneath and is twisted out to the left of the body of the zoospore (cf. fig. 17 for drawing).

FIG. 6. Stages in maturation of sporangial thallus. FIG. 6a (6 PM 6/30/51) Endobiotic apophysis filled with protoplasm; epibiotic sporangium with lipoidal globules more or less centrally disposed (cf. fig. 16a for drawing). FIG. 6b (44 hrs. later) Protoplasm of apophysis emptied into sporangium; lipoidal material organized with one globule for each zoospore initial (cf. fig. 16c for drawing). Note absence of rhizoids. Chl, chloroplast of host.

FIG. 7. Early developmental stages in formation of resting body. FIG. 7a. (5:30 PM 6/30/51) Encysted male cell—still with single lipoidal globule characteristic of the motile cell—attached to female thallus (cf. fig. 23a for drawing). FIG. 7b. (46 hrs. later) Plasmogamy has occurred; about one-half of the total protoplasm has passed out of the epibiotic part into the endobiotic, incipient resting body (cf. fig. 23b for drawing).

FIG. 8. Later stage in resting body formation, the epibiotic male and female parts completely emptied of protoplasm (cf. fig. 24 for drawing).

FIG. 9. Four mature resting bodies: the one to the right is out of focus, the bottom one is seen in surface view, the other two in optical sections. In all cases the warty appearance of the outer wall is apparent; the excentricity of the large lipoidal body is apparent in the lower two resting bodies. Note that many, healthy *Vaucheria* chloroplasts are still present.

PLATE 20

Chytridium sexuale sp. nov.

All figures × 1225 except for fig. 17, × 1905. Drawn from × 93 oil immersion lens.

FIG. 10. Zoospore before withdrawal of flagellum, at rest on healthy *Vaucheria* filament. In the thin layer of active host protoplasm between the cell wall and large central vacuole is seen a chloroplast in transverse view and two highly refractive globules.

FIG. 11. Germination of encysted zoospores. No penetration tube could be seen for the one to the right; the one to the left shows that the tube is considerably wider when penetrating the host wall than when in the host protoplasm; also, the old, encysted spore is no longer spherical but rather oval or oblong.

FIG. 12. The tip of the penetration tube has swollen, forming a rudimentary apophysis in which lipoid globules are accumulating; the distal end of the zoospore cyst has bulged out with the increased amount of protoplasm. There is no apparent difference between sporangial thalli and female thalli at this stage of development.

FIG. 13. Later stage in development of sporangium-producing thallus. The apophysis

to the left is almost completely filled with a single lipoid globule; to the right the apophysis is displaced as a result of host cytoplasmic streaming.

FIG. 14. Later stage in development of sporangial thallus; the entire complement of lipoid globules is shown.

FIG. 15. Exceptionally small, mature sporangial thallus, which produced four zoospores. *c*, callus.

FIG. 16. Maturation of a sporangial thallus (in optical section), and discharge of zoospores. FIG. 16a. (6:15 PM 6/30/51) Sporangium with lipoidal globules in "cluster stage" (cf. fig. 6a for photograph). FIG. 16b. (after 27 hrs.) Contents of apophysis emptied into sporangium, in which the lipoidal globules are in the "dispersion stage." FIG. 16c. (after 44 hrs.) After confluence of lipoidal material into rather large, similarly sized globules, one for each zoospore intial; pore between sporangium and apophysis apparently completely plugged (cf. fig. 6b for photo). FIG. 16d. (after 3 days, 21 hrs.) Zoospores escaping individually and remaining still in a loose cluster. Note that the sporangium has decreased slightly in diameter. FIG. 16e. (about 5 min. after initiation of discharge) The loosely clustered, discharged zoospores have unwrapped their flagella and are parting more or less simultaneously. Sporangium loosely filled with motionless zoospores which squeeze singly or in pairs through the slit in the sporangium wall, a flagellum wrapped around the body of each zoospore. FIG. 16f. (about 10 min. after initiation of discharge) Zoospores swimming within sporangium, one zoospore is moving amoeboidly through slit, another one, already outside, is pulling its flagellum free. Note excentric position of lipoidal globule in zoospore.

FIG. 17. Zoospores killed with fumes of 1% osmium tetroxide and stained lightly by adding a minute amount of crystal violet to the suspension of spores. *f*, whip-lash flagellum; *cyt*, cytoplasm (very lightly stained); *nc*, nuclear cap (highly refractive and lightly stained); *n*, nucleus (colorless); *l*, lipoidal globule (blackened by the osmium tetroxide in the labeled spore only).

FIG. 18. Tips of empty sporangia in which opercula were completely detached.

FIG. 19. Tips of empty sporangia in which opercula were not completely formed.

FIG. 20. One encysted and germinating male cell (thallus) and a male motile cell on the enlarged distal end of the epibiotic part of the female thallus.

FIG. 21. Three male cells encysted on the distally expanded, epibiotic part of the female thallus.

FIG. 22. Germinating male cells on small female thalli.

FIG. 23a. (5:45 PM 6/30/51) Same sexual stage of development as shown in fig. 22, but with female thallus further developed (cf. fig. 7a for photograph). FIG. 23b. (after 46 hrs.) Plasmogamy has occurred, and about one-half of the total protoplasm has passed out of the epibiotic part into the endobiotic, incipient resting body (cf. fig. 7b for photograph). FIG. 23c. (after 70 hrs.) Completion of inward movement of protoplasm.

FIG. 24. Similar stage to that shown in fig. 23c (cf. fig. 8 for photograph).

FIG. 25. Mature resting body in optical section (to right), and part of same resting body in surface view (to left); epibiotic female and male parts collapsed and disintegrating; host cytoplasm still streaming. Note callus around penetration tube.

FIG. 26. Mature resting body in optical section. Note small lipoidal globules around large, excentric one. *c*, callus; *d.chl*, disintegrating chloroplast.

FIG. 27. Accessory male cell, encysted and germinated, on empty female cyst.

PLATE 21

Chytridium stellatum comb. nov.

All drawings × 1215 from × 93 oil imm. obj. with the exception of Fig. 41, × 975 from × 70 water imm. obj.

FIG. 28. *Zygnema* filament with nine chytrid thalli. *e*, empty sporangial thallus (intramatrical apophysis and extramatrical, zoospore cyst persist, but the thin-walled part of the sporangium collapsed and is not discernible); *y*, young thallus; *r*, mature resting body;

s, top view of mature sporangium (zoospore cyst sticks out at bottom of sporangium). (× 516, using × 43 obj.; Wratten "M" plate, contact print.)

FIG. 29. Mature, intramatrical resting body in optical section, showing a large excentric lipoid globule with surrounding, tiny ones, and the glistening, deeply lobed outer wall (cf. fig. 41 for drawing). (× 700 using × 70 water imm. obj.; Wratten "M" plate, contact print.)

FIG. 30. Germination of encysted zoospore (to right) and early stages in formation of sporangial thallus.

FIG. 31 & 32. Later stages in development of sporangial thalli. Note tiny lipoid globules within rhizoids.

FIG. 33. Maturation and discharge of small sporangium. FIG. 33a. (8:50 PM 1/22/50) Small lipoid globules more or less evenly dispersed in cytoplasm of extramatrical, full-sized sporangium; apophysis empty. *hw*, host wall. FIG. 33b. (after 2 hrs.) After confluence of lipoid material into six large globules, one for each zoospore. Note vacuolate protoplasm. FIG. 33c. (after 4 hrs.) Discharging sporangium. Note collapse of thin-walled part and persistence of old-zoospore part. FIG. 33d. (after 4 hrs. 30 secs.) Zoospores separating slightly and extending their flagella. *op*, operculum. FIG. 33e. (after 4 hrs. 1 min.) Zoospores in process of rapidly swimming away. Note excentric position of lipoid globule in zoospore.

FIG. 34. Small, exceptional thallus with apophysis as well as sporangium developing extramatrically.

FIG. 35. Germinating zoospore, apparently unable to penetrate *Zygnema* wall, with extramatrical rhizoids. Note that rhizoids are blunt-tipped. *c*, callus.

FIG. 36. Discharge of sporangium, which is in a similar condition to the one at left(*s*) in fig. 28. A few minutes after the dispersal of zoospores the sporangium wall collapsed. FIG. 36a. (10:25 PM 1/20/50) Sporangium in optical section, with old zoospore cyst and host cell wall also indicated. Note large vacuole in discharge papilla. FIG. 36b. (1 hr. 51 mins. later). *op*, operculum.

FIG. 37. Young thallus which, because of the emptying of the contents of the encysted zoospore into the intramatrical swelling, is presumably an incipient stage in resting body formation.

FIGS. 38 & 39. Later stages in resting body formation.

FIG. 40 & 41. Mature resting bodies showing the thin inner wall and the outer wall with pointed, blunt and lobed excrescences. FIG. 41. To right—optical section showing that the inner wall completely isolates the resting body protoplast. To left—surface view on tips of excrescences.

PLATE 19

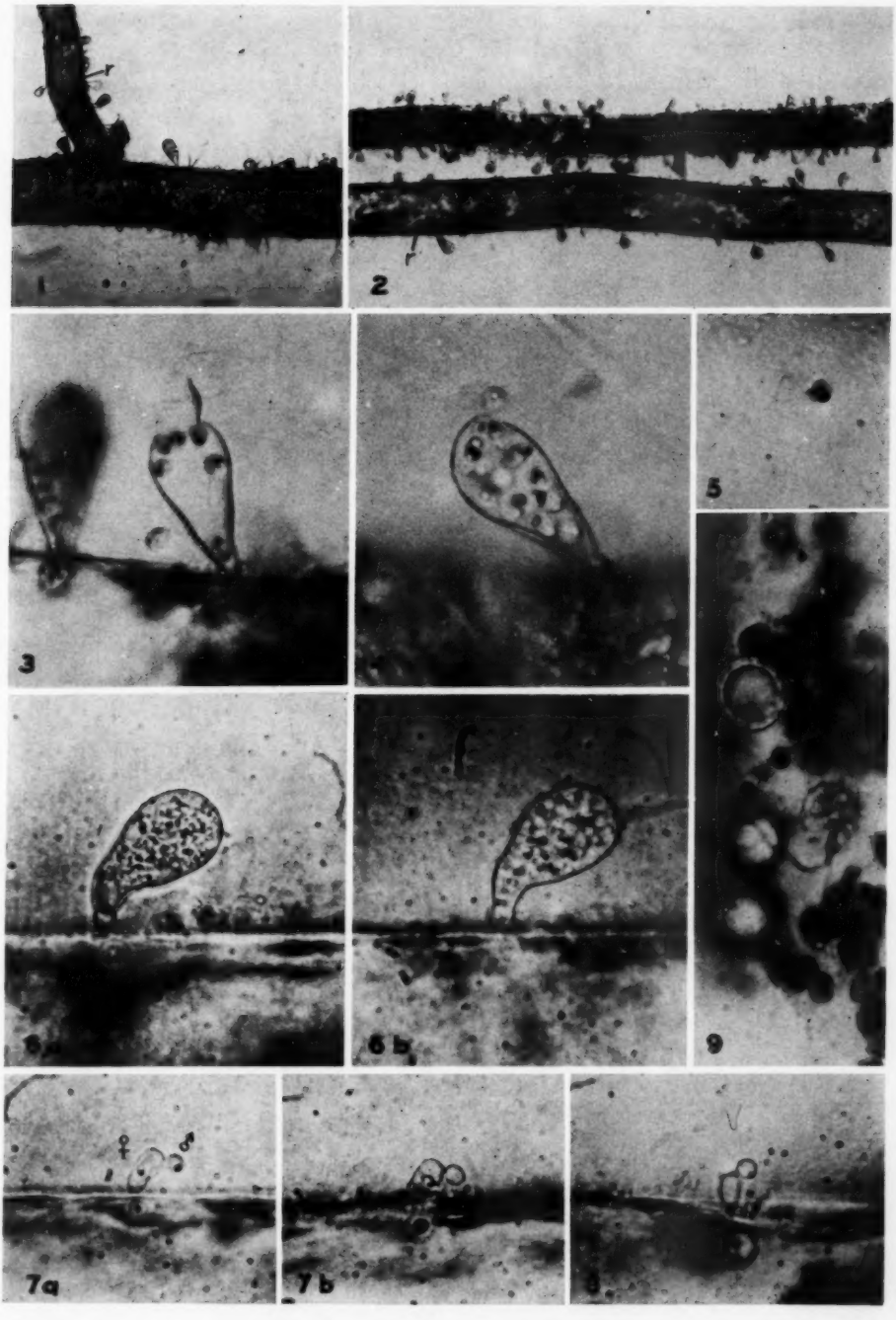
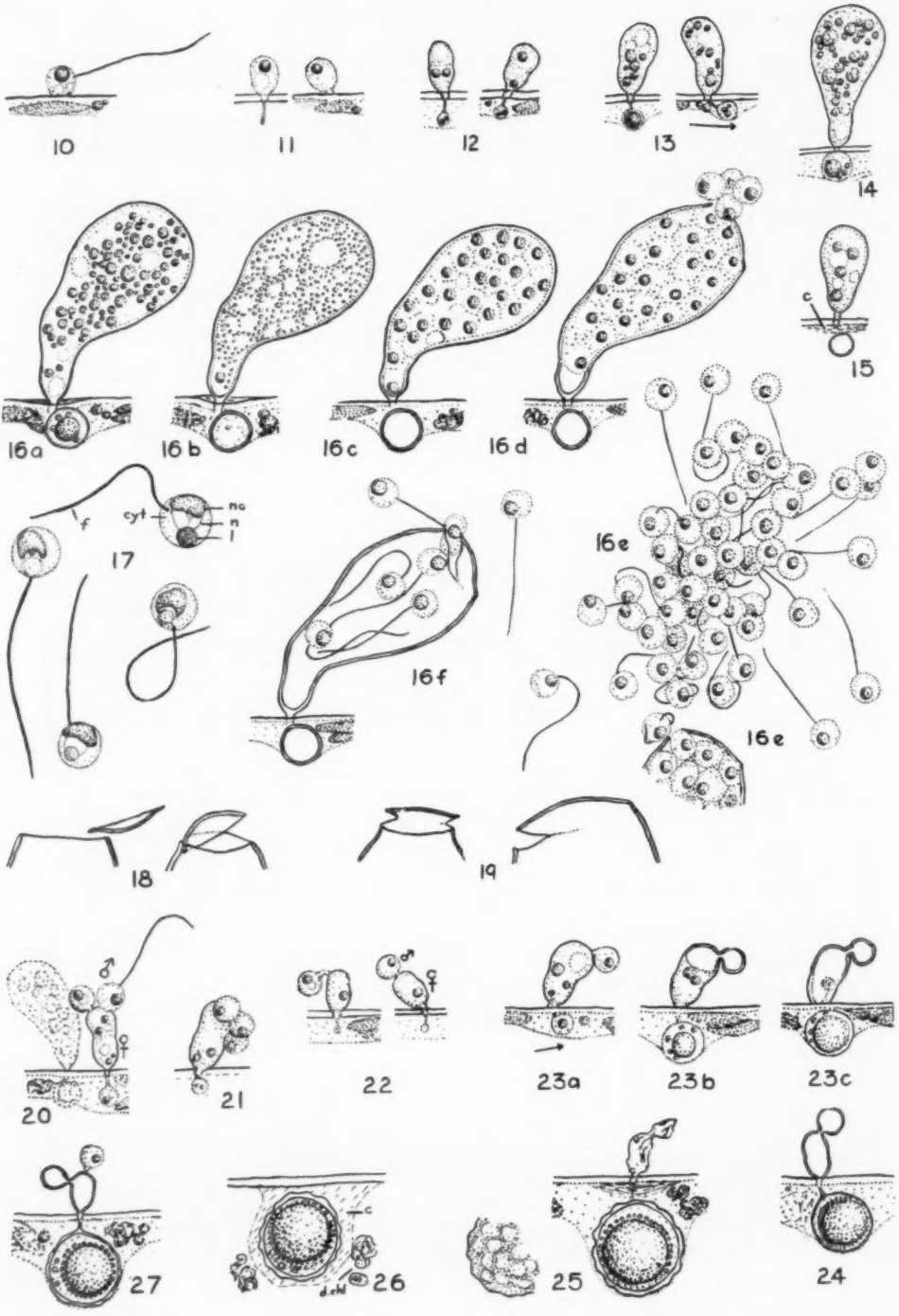


PLATE 20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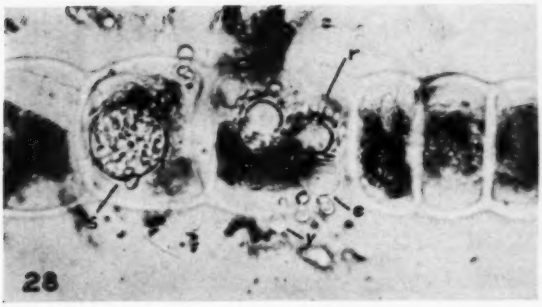
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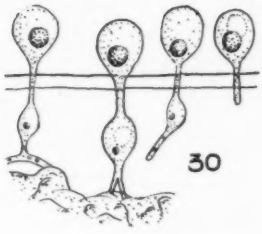
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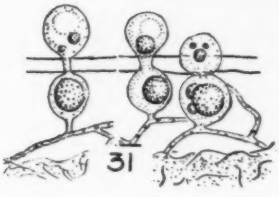
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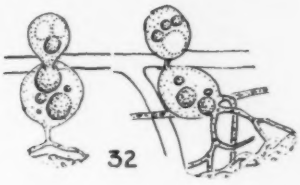
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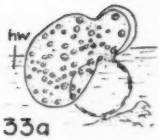
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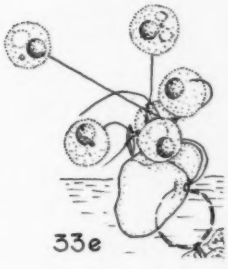
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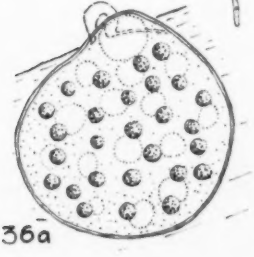
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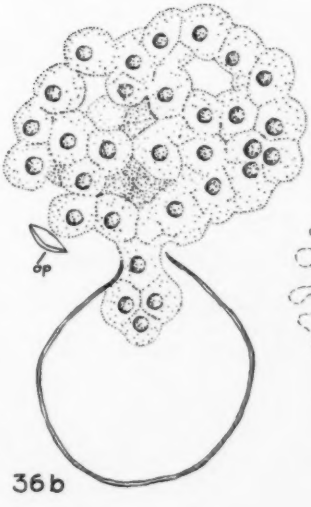
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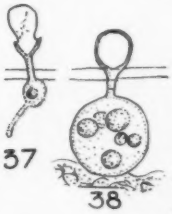
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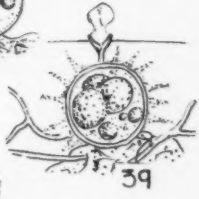
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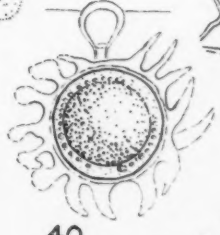
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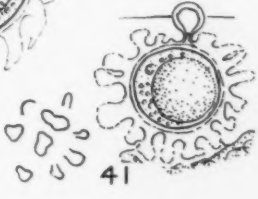
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